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SPACE-CABIN ATMOSPHERES

Part I—Oxygen Toxicity

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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Part I—Oxygen Toxicity

A literature review by
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Foreword

THIS REPORT, previously published as NASA Technical Note D–2008 in August 1963, is Part I of a study on *Space-Cabin Atmospheres*, conducted under sponsorship of the Directorate, Space Medicine, Office of Manned Space Flight, National Aeronautics and Space Administration. Part II, "Fire and Blast Hazards," is available as NASA SP–48. Future parts of this study will be: Part III, "Physiological Factors of Inert Gases," and Part IV, "One- versus Multiple-Gas Systems."

This document provides a readily available summary of the open literature in the field. It is intended primarily for biomedical scientists and design engineers.

The manuscript was reviewed and evaluated by leaders in the scientific community as well as by the NASA staff. As is generally true among scientists, there was varied opinion about the author's interpretation of the data compiled. There was nonetheless complete satisfaction with the level and scope of scholarly research that went into the preparation of the document. Thus, for scientist and engineer alike it is anticipated that this study will become a basic building block upon which research and development within the space community may proceed.

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Deputy Director, Space Medicine
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Introduction

> . . . But, perhaps we may also infer from these experiments, that though dephlogisticated air might be very useful as a medicine, it might not be so proper for us in the usual healthy state of the body; for, as a candle burns out much faster in dephlogisticated air than in common air, so we might, as may be said, live out too fast, and the animal powers be too soon exhausted in this pure kind of air. A moralist, at least, may say that air which nature has provided for us is as good as we deserve. . . .
>
> *Priestley, 1775* [135]

As soon as the first oxygen was made available for study, the toxic potency of this gas was recognized. The classical reviews by Stadie, Riggs, and Haugaard in 1944 [151] and by Bean in 1945 [8] cover in detail most of the work to this time. An unpublished brief review by Snapp and Adler [149] summarizes the more significant features of oxygen toxicity to the 1948 period. Most of the signs and symptoms and potential mechanisms of oxygen toxicity were well worked out by this time. Subsequent studies have been related primarily to the mechanism of oxygen toxicity and to studies of unusual environmental conditions employing high oxygen concentrations.

The present review is limited to the data on oxygen toxicity that are important in the analysis of space-cabin atmospheres. Of chief concern are the effects of oxygen at pressures below 1 atmosphere. Oxygen at higher pressures is discussed only to help elucidate mechanisms of toxicity in the space-cabin environments. Chapter 1 covers the molecular mechanism of oxygen poisoning. Subsequent chapters treat oxygen toxicity and mechanisms in animals and in man; the role of oxygen in atelectasis, blast effects, and the space radiation problem; drug therapy against oxygen toxicity; and consideration of oxygen toxicity in the selection of a space-cabin atmosphere.

CHAPTER 1

Molecular Mechanisms

PHYSICAL CHEMISTRY OF OXYGEN MOLECULE

A TRUE EVALUATION of the gross physiological responses to high oxygen tensions requires an understanding of the biochemical interactions of oxygen at a molecular level. The peculiar properties of the oxygen molecule are derived from its unusual electronic configuration. Pauling [129] was first to point out the paramagnetic nature of oxygen resulting from its two unpaired electrons. Pauli's exclusion principle requires that two electrons in the same orbital have opposite spins with neutralization of magnetic moments. Paramagnetism results from the magnetic moments presented by unpaired electrons of oxygen and free radicals.

Figure 1 demonstrates the electrons at the end of the axes of the P orbitals (X, Y, and Z). The two unpaired electrons are indicated by the arrows at P_Y and P_Z. The electrons of oxygen can form two 3-electron bonds (:O:::O:), but it is thought that oxygen gets its paramagnetic behavior from the presence of two unpaired electrons.

By virtue of its unusual electronic structure, oxygen has a high oxidizing potential which endows it with its properties as the ultimate oxidizing agent for the maintenance and, as we shall see, destruction of many living systems. The destructive oxidizing capacity of the oxygen molecule is kept in check by several peculiar aspects of its own structure and that of living systems with which it interacts.

Oxygen (O_2) is useful as a potential energy source because it is in reality a rather "sluggish" oxidizing agent which gives it an "energy storage" function. In 1940 Gorin [75] pointed out that the sluggishness of oxygen is probably due to the fact that it has to be activated to the free-radical state for its intracellular role. Michaelis [112] in 1949 postulated that the reduction of oxygen proceeds through several univalent steps which would imply free-radical intermediates. Using the electron magnetic resonance techniques of Sogo and Tolbert,[150] Commoner et al.[34] in 1957 demonstrated free radicals as probable intermediates in oxidation-reduction in chloroplast systems.

Szent-Györgyi [155] has recently reviewed the analogy between semiconductor systems and the conduction of electrons along proteins and oxidation-reduction enzymes of biological systems. Gerschman [62] has reviewed the possible reaction of oxygen with hydrogen to form the hyperoxal (hydroperoxo) free radical $HO_2\cdot$ or $H\cdot + OH\cdot$ with unpaired electrons (fig. 2). (The dot after a molecule represents a free radical capable of attacking many types of bonds.)

Gerschman [62] postulated that the activation energies predicted for the reduction of oxygen in univalent free-radical steps would tend to act as energy barriers preventing rampant oxidation of cellular components by free oxygen. (See fig. 3; ΔF_0 represents free-energy change.)

Once in active free-radical form, oxygen can react with many cellular components. The

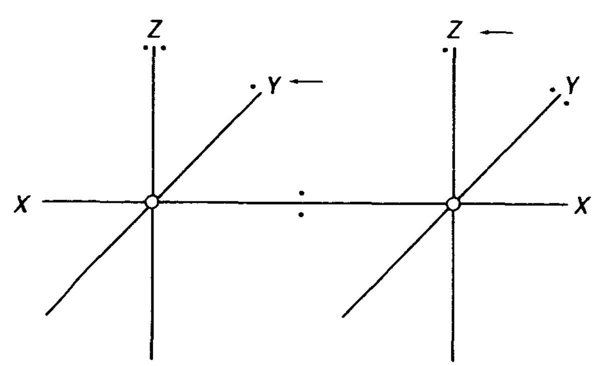

FIGURE 1.—*Electronic configuration of molecular oxygen O_2.* (AFTER GERSCHMAN.[62])

capacity of oxygen to partake in chain reactions with organic systems has been beautifully reviewed by Walling.[163] The initiation and prolongation of the chain process is reviewed subsequently. Free radicals, of course, may be generated by ionizing radiation as well as by metabolic processes. The interaction between oxygen effects and radiation has been known for years as "the oxygen effect" and is discussed in a later section. Effects of ozone toxicity appear to follow the same general free-radical mechanisms (Davis [41]).

ANTIOXIDANT DEFENSES

The defense of cellular systems against free radicals generated by oxidative processes is still poorly understood. It is very possible that somehow the aging processes of the entire body may well represent the progressive deterioration of antioxidant defense. The relation of destructive oxidative processes to the aging of red blood cells is discussed in a subsequent section. As will be seen, the generation of hydrogen and electrons by the degradation of carbohydrates and other energy sources contributes to antioxidant defense. The reduced form of triphosphopyridine nucleotide (TPNH) which finally results from these reactions reduces in turn the glutathione, cysteine, and other active reducing compounds within the cell. Other mechanisms also contribute to the antioxidant defense.

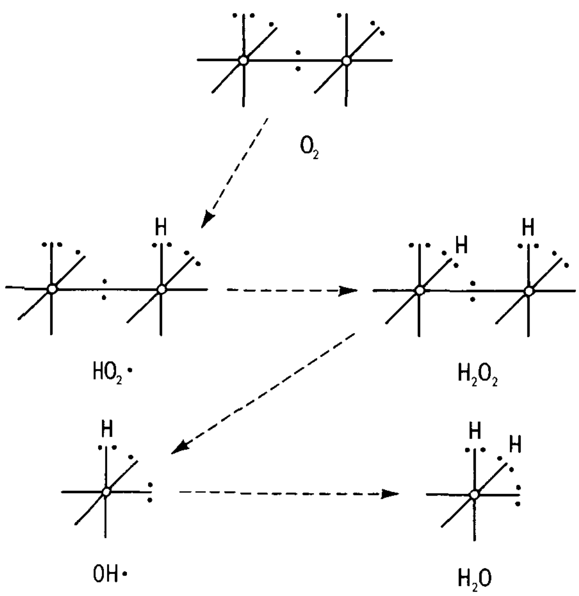

FIGURE 2.—*Hydrogen-oxygen species.* (AFTER GERSCHMAN.[62])

Chance [28] has recently pointed out the peculiar role of the terminal cytochromes as buffers for the oxidative system. Reductive changes in the terminal oxidases and proximal members of the respiratory chain occur at oxygen concentrations exceeding the critical level based upon cellular respiratory activity. The overabundance of terminal oxidases allows them to be oxidized by molecular oxygen and to leave, nevertheless, an adequate amount of the reduced form to carry on respiratory processes without measurable changes in the respiratory rate. By providing a storage of bound oxygen, this system probably buffers the cell in anoxic states as well.

Gerschman et al.[65] (1955) and Taylor [157] (1956) have demonstrated the role of vitamin E and the α-tocopherols as antioxidants in the cell. Indeed, some symptoms of vitamin E deficiency are probably those of toxicity to 0.2 atmosphere of oxygen, the normal sea-level condition. Animals deficient in vitamin E are very sensitive to high-oxygen environments.[110] The importance of this concept will become clearer in the discussion of recent experiments in space-cabin simulators.

Bacteria have been known for years to have antioxidant defenses. Porter [134] demonstrated that obligate anaerobes die in the presence of oxygen because they lack catalase. This is indeed the rationale for the new OHP (oxygen at high pressure) treatment of tetanus. Annear and Dorman [2] and Gordon et al.[74] demonstrated that hydrogen peroxide was indeed the lethal factor. High oxygen pressures can actually cause mutations,[55] possibly through the depolymerization of deoxyribonucleic acid (DNA)[70] via the peroxide or free-radical mechanism. This suggests that genetic stability depends on adequate antioxidant defense.

FREE-RADICAL CHAIN REACTIONS

It appears that oxygen toxicity and damage by ionizing radiation proceed by similar mechanisms. Both involve free-radical mechanisms. Excess levels of free radicals start chain reactions typical of auto-oxidation processes. This concept is outlined below, where RH is a normal carbon-hydrogen bonded organic molecule, R· is a normal active biological free-radical intermediate, and RSH is a normal biologically active thiol group on an organic molecule.

I. Initiating steps

A. Ionizing radiation

1. Indirect (via water)

$$H_2O \xrightarrow{h\nu} H_2O^+ + E^-$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH\cdot$$

$$E^- + H_2O \rightarrow OH^- + H\cdot$$

2. Direct effect on biological molecules

$$RH \xrightarrow{h\nu} R\cdot + H\cdot$$

B. Biological reduction of O_2

$$O_2 \xrightarrow{H} HO_2\cdot \xrightarrow{H} H_2O_2 \rightarrow OH\cdot + H_2O \xrightarrow{H} 2H_2O$$

C. Oxidation of R· by O_2

$$R\cdot + O_2 \rightarrow RO_2\cdot$$

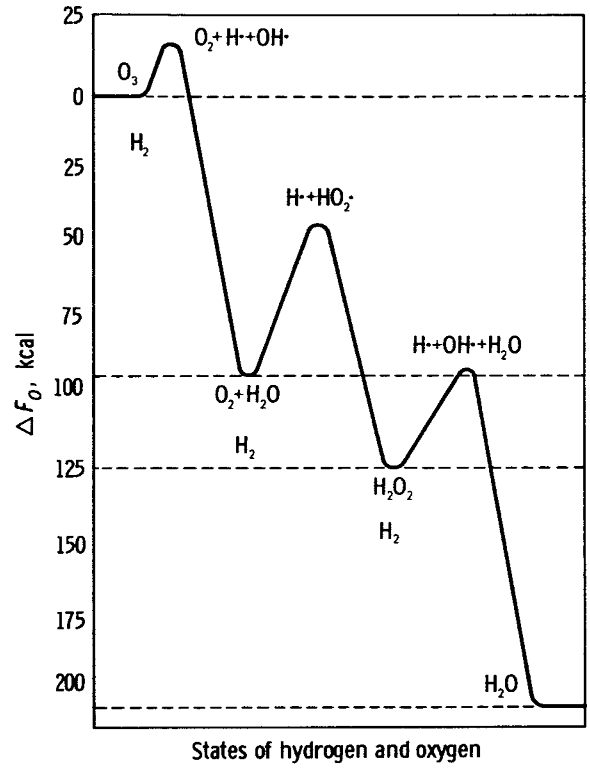

FIGURE 3.—*Reduction of oxygen by hydrogen. Dashed lines refer to stable or quasi-stable states.* (AFTER GERSCHMAN.[62])

II. Damaging steps (chain reactions)

A. $RH + HO_2\cdot \rightarrow R\cdot + H_2O_2 \rightarrow$ Step I*C* $\rightarrow$ Chain reaction

B. $R'O_2\cdot + RH \rightarrow R'O_2H + R\cdot \rightarrow$ Step I*C* $\rightarrow$ Chain reaction

C. $RSH + HO_2\cdot \rightarrow RS\cdot + H_2O_2 \begin{cases} \rightarrow \text{Step I}C \rightarrow \text{Chain reaction} \\ \rightarrow \text{Step III}C \rightarrow \text{Permanent damage or chain termination} \end{cases}$

III. Chain-terminating reactions (permanent damage or protection by stopping free-radical chain)

A. $R'O_2\cdot + R\cdot \rightarrow R'O_2R$

B. $R\cdot + R\cdot \rightarrow RR$

C. $RS\cdot + RS\cdot \rightarrow RSSR$

The propagation of free-radical chain reactions is characteristic of both types of insult. As Gerschman et al.[63] pointed out, effects of the reducing agents cysteamine, glutathione, and thiourea protect mice against radiation as well as against oxygen, even though at lower oxygen concentrations they may actually potentiate the oxygen effect. In the latter case, they probably are acting as pro-oxidants in presenting a cell with a thiol compound which is converted by O_2 to $RS\cdot + HO_2\cdot$ (step II*C* above). The sulfhydryl compounds cystamine and aminoethylisothiuronium (AET) act similarly.

Cobalt has been shown by Gilbert et al.[71] to destroy hydrogen peroxide, and by Gerschman et al.[63] to protect against 1 atmosphere of oxygen as well as against ionizing radiation.[126] Obligate anaerobes can actually grow in the presence of oxygen when cobalt is added to the medium.[42] The metal complexing agents ethylenediaminetetracetic acid (EDTA) and diethyldithiocarbamic acid (DEDTC) have been shown by many investigators[63,85] to protect intact animals and enzymes against both irradiation and high oxygen tensions. These agents possibly chelate out the heavy metals such as copper which catalyze peroxy free-radical reactions.

Thiols are probably not the only compounds involved in the generation of reactive free radicals. During the past few years there has been an increased interest in the effects of peroxidation products of lipids on biological systems.[106] Several investigators have demonstrated that lipid peroxides may be responsible for some of the effects of radiation.[91,101,118] In his study of OHP, Wollman [173] demonstrated a significant increase in cerebral lipid peroxides of OHP with no significant changes in $-SH$ groups. Becker and Galvin [9] recently confirmed these findings, but noted that there is no correlation of the peroxides with convulsive activity of OHP cerebral toxicity. No lipid peroxide elevation was noted in oxygen partial pressures of 1 atmosphere or less. It is still possible, however, that focal increases in lipid peroxides may indeed play a role in the destruction of red blood cells and alveolar membranes in the lower toxic p_{O_2} range.

BIOLOGICAL VARIABILITY

Upon consideration of the mechanism of action, the biological variability in the effects of high oxygen concentrations, depending on concentration, species, protective agents, and so forth, becomes more rational. The survival equation of Williams and Beecher,[168] $T=aP^{-b}$, where T is time in hours, P is pressure (atm) of oxygen, and a and b are empirical constants, has been found by Gerschman et al.[63] to be valid for $a=102$ hours and $b=2.73$ from only 1 to 10 atmospheres of oxygen. A big change appeared at 0.7 to 1.0 atmosphere. If oxygen does produce active free-radical intermediates, a sudden increase in chain reaction rates would be expected at a very specific concentration range. This would be evidenced by a sudden increase in sensitivity to oxygen pressure. This critical-dose effect is also seen in X-irradiation of mice.[98] The concentration of antioxidants and chain-terminating thiol compounds [163] at critical cellular sites would, therefore, be expected to determine the specific gross pathological physiology. Especially in dealing with the lower concentrations of oxygen (<1 atm), one would expect gross irregularities in effect from small changes in oxygen concentration and cellular environmental factors.

The possibility of sensitizing agents (to be discussed later) further complicates the picture. Many of the "target organ" variabilities and moderating factors in oxygen toxicity are discussed below. Accepting the role of oxygen as an initiating agent in generating free radicals, what are the actual target molecules (RH and RSH) of these agents within the cell? They appear to be the enzyme systems and nucleic acids. The effects of oxygen on these systems will now be discussed.

CRITICAL TARGET MOLECULES

Early studies of the effect of high oxygen tensions on enzymatic activity implicated the oxidase-dehydrogenase enzyme group as the optimum intracellular target.[8,44,59,151] Both in tissue slices and in isolated enzyme systems, oxygen appears to be inhibitory. The generation of reducing agents by enzyme systems of the carbohydrate degrading systems or even exogenous reducing agents frequently relieved the oxygen inhibition. Often the coenzymes themselves were not the prime targets.[44] Metal ions such as those of manganese, cobalt, magnesium, and calcium, which preserved the general glycolytic pathway for generation of reducing agents, were effective in reducing damage.

The pyruvate oxidizing system appeared quite sensitive. The dehydrogenase part of the succinoxidase system was irreversibly damaged in the brain preparations, and the cytochrome system was only weakened after longer exposure. Lactic and malic dehydrogenases were weakly affected. Triosephosphate dehydrogenase (only in the absence of cozymase) was oxygen sensitive, as was choline oxidase, another $-SH$ brain enzyme. The brain lactic and malic dehydrogenases, flavin adenine dinucleotide (FAD) systems, catalase, and hexokinase were not sensitive to oxygen. These experiments appear to invoke the reactions:

$$RSH + O_2 \rightarrow RS\cdot + HO_2\cdot$$

and

$$RSH + HO_2\cdot \rightarrow RS\cdot + H_2O_2$$

with possible irreversible changes to

$$RS\cdot + RS\cdot \rightarrow RSSR$$

where RSH represents thiol apoproteins or coenzymes.

The diverse enzymes which utilize the coenzyme A system for "2 carbon" transfers appear to be good targets for oxygen. It has been shown [68] that β-mercaptoethanolamine (β-mercaptoethylamine), a component of coenzyme A, will protect mice against oxygen poisoning and irradiation. The pyruvic oxidase system which is central in the metabolic cycle and uses coenzyme A is, indeed, very sensitive to oxygen,[44] especially in the presence of cupric ions.[85]

The lipid peroxides that have been discussed as possible intermediates in the free-radical chains have been recently shown to inactivate specific enzymes. These compounds have been studied primarily for their role in radiation damage,[91, 118] but their effects are, of course, also pertinent to the oxygen toxicity problem. Bernheim et al.[17] have demonstrated that the oxidation of mitochondrial fatty acids inactivates succinoxidase, cytochrome oxidase, and choline oxidase. Tappel and Zalkin [156] and Wills [170] confirmed the effects of these peroxide compounds on enzymes. These investigators have suggested that since the mitochondrial cytochromes and other hematin compounds are the most active peroxidation catalysts in animal tissues, the unsaturated fatty acids of the mitochondria are the most probable intermediates in oxygen inactivation of the respiratory chain.

A recent finding of Dixon et al.[45] indicates that the enzyme most sensitive to oxygen is cytochrome C reductase of the pig heart. These investigators found, in purified preparations, a half-life of 6 minutes at 38° C and 1 atmosphere, as compared to the most sensitive enzyme of Dickens,[44] succinic dehydrogenase, with a half-life of 3 hours under the same conditions.

Of interest is the mechanism of this oxidative inactivation. The apoprotein itself is oxidized; critical is the binding, not the oxidation of the prosthetic flavin group. Apparently, oxidation of the binding site prevents flavin attachment. Glutathione does not protect, nor do CN^-, Versene, or dipyridyl groups, even though this enzyme, contains iron. This inactivation mechanism is the clearest picture we have of a specific molecular effect. Destruction of this enzyme may be, indeed, the critical factor in oxygen action, the other enzymatic defects merely changing the redox potential of the intracellular environment to accelerate the oxidation of this rate limiting step.

It seems probable that the movement of electrons in neighboring molecules and in critical protein that composes cellular structural elements may also be interfered with by oxygen.[155] Any enzyme may, therefore, be potentially affected *in vivo*. As has already been mentioned, nucleic acid molecules may also be targets for the free-radical reactions.

The *in vivo* cellular environment, it must be remembered, does have antioxidants and reducing metabolites, which alter the oxygen effects from those detected *in vitro*. The array of the multienzyme systems on the matrix of mitochondria[79] may indeed protect terminal respiratory enzymes against oxygen damage. With the isolated pure compound cytochrome C, Theorell [158] demonstrated that the peptide helix so encloses the hemin plate as to completely shield this active site from oxygen, but not from electrons. Similar shielding from oxygen may be present in many of the enzyme systems of living cells.

The protective effect of hypophysectomy[25] and adrenalectomy, and the augmentation of oxygen poisoning by very high doses of adrenal cortical hormones, bespeak the role of these hormones in control of cellular energetic reactions.[66] The multiple sites of action of adrenal cortical hormones only confuse the picture in our attempt to understand the molecular basis of oxygen toxicity in the intact animal. Yet, the adrenal has been demonstrated to control the level of cerebral lipid peroxides in OHP.[9] There have been many other studies of vitamin deficiency and other metabolic stress in relation to oxygen toxicity, but none appear to have shed any light on specific molecular mechanisms. These are discussed in Chapter 7.

FOREIGN INTEREST

The Russians have been doing work on free-radical interrelations between oxygen toxicity and radiation exposure. They have been stressing free-radical reactions and have been looking for "oxygen content in tissue" as a measure of the effectiveness of the $-SH$ drugs against radiation. A typical example is a recent paper on antiradiation effect of thiourea and monothiols,[76] reviewed in Chapter 6 of this report. The Russians appear to be continuing this oxygen-radiation tack.[77, 97, 98, 102, 125]

The Russians seem to have an interest in oxygen at high pressure (OHP), probably for submarine, scuba, and therapeutic purposes. Recent studies of brain metabolism in the 3- to 6-atmosphere range revealed a release of large quantities of ammonia which were lowered by administration of arginine. The researchers postulated that oxygen breaks down brain protein, releasing NH_2 groups, and the arginine scavenges it in the form of γ-amino butyric acid (GABA). It is of interest that Wood and Watson [174] of Toronto have recently demonstrated that GABA protects animals against the convulsions of OHP. The formation of glycogen in the brains of rabbits exposed to OHP is also being studied.[22] The work of Dickens [44] mentioned previously suggests that decreased glycolysis may be the ultimate causative factor in this glycogen increase.

It would thus appear that the molecular basis of oxygen toxicity may be related to the capacity of oxygen to (1) initiate free-radical reactions which interfere with enzymatic activity by direct reaction with apoproteins or coenzymes, and (2) modify the general redox potential within the cell and inhibit critical reactions. The signs, symptoms, and pathological physiology of oxygen toxicity, especially in the <1 atmosphere pressure range, appear sensitive to small changes in oxygen tension and to the metabolic state of the cells.

CHAPTER 2

Effects of High Oxygen Tension in Animals

IN GENERAL, it appears that oxygen toxicity falls into two target-organ classes: at <2 atmospheres, the respiratory tract is hit; at >2 atmospheres, the central nervous system is the key organ. In this review, the problems at <1 atmosphere will be emphasized.

Recent reviews of the <2 atmosphere range have been presented by Mullinax and Beischer [117] and DuBois.[49] They indicate that slight variations in test conditions from experiment to experiment are probably significant in the pathological physiology. This, of course, would be expected from the critical oxygen-tension factor postulated above for the <1 atmosphere condition. Great pains will, therefore, be taken to emphasize the critical details of the experiments.

TENSIONS OF 0.75 TO 1 ATMOSPHERE

Smith [148] studied the effects of 0.7 to 0.8 atmosphere (600 to 760 mm Hg) of oxygen on birds, mice, rats, and guinea pigs. He found that after 4 days the animals died with signs of "early stages of pneumonia" and hyperemia of the lungs and other organs. Elevation of oxygen pressure to higher levels hastened their death. At 0.4 atmosphere (306 mm Hg), no such pulmonary changes were found. Stadie et al.[151] and Bean [8] confirmed these results.

Clamann and Becker-Freyseng [10, 31] exposed 50 assorted animals to 0.80 to 0.87 atmosphere oxygen (601 to 607 mm Hg) for 7 days and found, besides severe pulmonary edema, mediastinal edema and pleural exudates. Cats and rabbits showed marginal emphysema. Lungs were hyperemic; alveoli were edematous, filled with red and white blood cells, and lined with a debris-filled membrane. This membrane adhered to vascular walls, extended into bronchioles, and appeared fibrinous in nature. Employing similar oxygen conditions, Pichotka [133] and Liebegott [104] described the same picture, as did Ohlsson.[122] Paine et al.[123] described similar findings in dogs in 0.75 to 1 atmosphere (570 to 760 mm Hg), but signs of right-sided heart failure were more evident.

Penrod [130, 131, 132] pointed out that rats and guinea pigs have endemic lung diseases which complicate pathological studies and suggested that cats be used. He found that by cannulating one bronchus and occluding the other during administration of 100 percent oxygen at several atmospheres for 3 hours, he could produce pathology similar to that described above in the open lung, but not in the occluded lung. He suggested that this result indicates a direct effect on the alveolar membrane and eliminates the hypothesis of a blood-borne toxin. Atelectasis is also found in the blocked lung. An oxygen pressure of 3 atmospheres for 4 hours tends to cause mucoid plugs in bronchioles and secondary atelectasis in cats. Repeated exposures to air during OHP reinflates the lung and decreases damage to the central nervous system by OHP. Positive-pressure breathing also alleviates the signs of lung damage. A recent study by Weir et al.[165] confirms all the above animal findings.

In a study [145] of lung pathology resulting from oxygen toxicity (1 atm), the electron microscope showed that the mitochondria became vacuolated. Treciokas,[161] however, suggests that these vacuolated structures are found in normal lungs and are probably not early signs of oxygen damage. The latest electron-microscope study of oxygen toxicity [27] in mice exposed to 1 atmosphere of oxygen (95 to 100 percent) and 80 to 90 percent humidity revealed, after 3 to 6 days, an apparent patchy thickening of the alveolar wall due either to hypertrophy or fluid accumulation in the cells. The splitting of basement membrane and fluid vacuoles between endothelial cells and membrane were also seen. This damage is probably responsible for the passage of fluid from blood into the alveoli, though occasional fluid-filled alveoli were seen without these changes. Macrophages were occasionally seen to have the "mitochondrial vacuolization" of Schulz,[145] as were alveolar cells. These are usually present in normal lungs and may be fixation artifacts. The membranes in the alveoli contain an atypical fibrin similar to human "hyaline membrane" disease. No characteristic bacterial flora was seen.

Cells other than those in the lung have been shown to be damaged by oxygen at <1 atmosphere. Noell[121] has recently demonstrated that the electroretinograph (ERG) potentials are attenuated and disappear in rabbits exposed to high concentrations of oxygen at 1 atmosphere total pressure. Time of disappearance and rate of decline are dependent on actual oxygen pressure. The visual cells of the retina are sensitive to oxygen concentrations at 1 atmosphere or less at times when no other sign of systemic oxygen toxicity is evident. The following times were adequate for destruction of visual cells at 1 atmosphere total pressure: all animals exposed for 40 hours at 100 percent oxygen, 50 percent of animals in 4 days at 80 percent oxygen, 50 percent of animals in 7 days at 55 to 60 percent oxygen, and no animals in 12 days at 50 percent oxygen. The rabbit appears unusually sensitive, but young rabbits were more resistant than old. In mice, rats, and cats, death of the animal from other organ sensitivities occurred before cell death was visually evident.

The role of carbon dioxide in oxygen toxicity was studied by Lambertsen et al.[99] The early high carbon dioxide levels in tissues reported by others in the past were shown to be artifacts of the method of measurement. No true rise in carbon dioxide was found in dogs, rabbits, or cats until the onset of convulsions resulting from an environment of 3 to 4 atmospheres of oxygen. The hypothesis that the hemoglobin-carbon dioxide transport defect initiated by OHP is the primary cause of death was thereby discredited. Primary pulmonary damage and convulsive activity were thought to be the prime causes of carbon dioxide elevation. The potentiating effects of 2 to 3 percent carbon dioxide on the pulmonary damage from oxygen toxicity in the <1 atmosphere range has been discussed by Ohlsson.[122]

It can be seen that most of the pathology in animals exposed to the range of p_{O_2} from 0.75 to about 1 atmosphere involves the lung. The effect appears to be directly on the alveolar walls and leads to a cyanotic death. Other organs may well be involved at a chemical level, but there is little evidence of gross pathology. Rabbits appear to have retinas which are especially sensitive. It is possible that gross pathology would be seen in other organs if the animals would live long enough with their pulmonary insult. Carbon dioxide retention appears to be an aggravating factor rather than a prime force. The carboxyhemoglobin mechanism which was once in vogue appears to be only a complicating factor in the cerebral as well as the pulmonary aspects of oxygen toxicity. Atmospheric carbon dioxide in the 2 to 3 percent range does hasten death from pulmonary damage.

TENSIONS OF 0.20 TO 0.75 ATMOSPHERE

The studies described in the preceding section were at oxygen tensions from 0.75 to 1 atmosphere. Little work has been done at tensions in the 0.20 to 0.75 atmosphere range. A much overlooked study performed by Campbell[24] in 1927 sheds some light on the problems that currently face us. Campbell exposed cats, rats, mice, cavies, monkeys, and rabbits to oxygen tensions in the range of 0.6 to 3 times that in air, up to 59 days, with many environmental and physiological parameters under constant surveillance. Monkeys, cavies, rats, and mice tolerated an oxygen pressure of 420 mm Hg (60 percent oxygen) for these prolonged periods without symptoms or excessive weight loss, except for the cavies, which lost weight. Cats, however, when exposed to oxygen at only 300 mm Hg (40 percent) showed symptoms of sleepiness and loss of appetite, and lost weight.

Pathological examinations of cat lungs showed "collapse and few catarrhal cells." This finding is of interest in that Penrod [131] later reported that cats under OHP have a tendency to produce mucoid plugs in small bronchioles and suffer atelectasis. Most animals demonstrated hemoglobin depression of 30 percent while cats showed slight rises in hemoglobin (fig. 4). The elevated hemoglobins and white blood cells in cats may indicate a tissue anoxia from atelectatic processes in the lungs. Oxygen tension in the abdominal cavities of cats was indeed elevated to a lesser degree than in the other animals (fig. 5).

The animals (cats not measured) all showed a normal or slightly depressed reticulocyte count. Only one reticulocyte count at an unknown point in the experiment is reported. A prussian blue study of the spleens of rats and mice showed greater pigment deposition, suggesting an excessive rate of breakdown of red blood cells. Thus, cells seemed to be hemolyzing with an inadequate reticulocyte response. The hemoglobin content per cell was slightly elevated in these animals. Carbon dioxide tensions in the abdominal cavity of all the animals were slightly elevated. The findings of possible hemolysis of red blood cells in the presence of elevated oxygen tensions in the tissues are of particular interest and will be discussed later.

In 1960 MacHattie and Rahn [108] studied the effect of nitrogen-free environments in the growth and reproduction of mice. The animals were maintained for 51 days in an atmosphere of oxygen at a total pressure of 197 mm Hg,

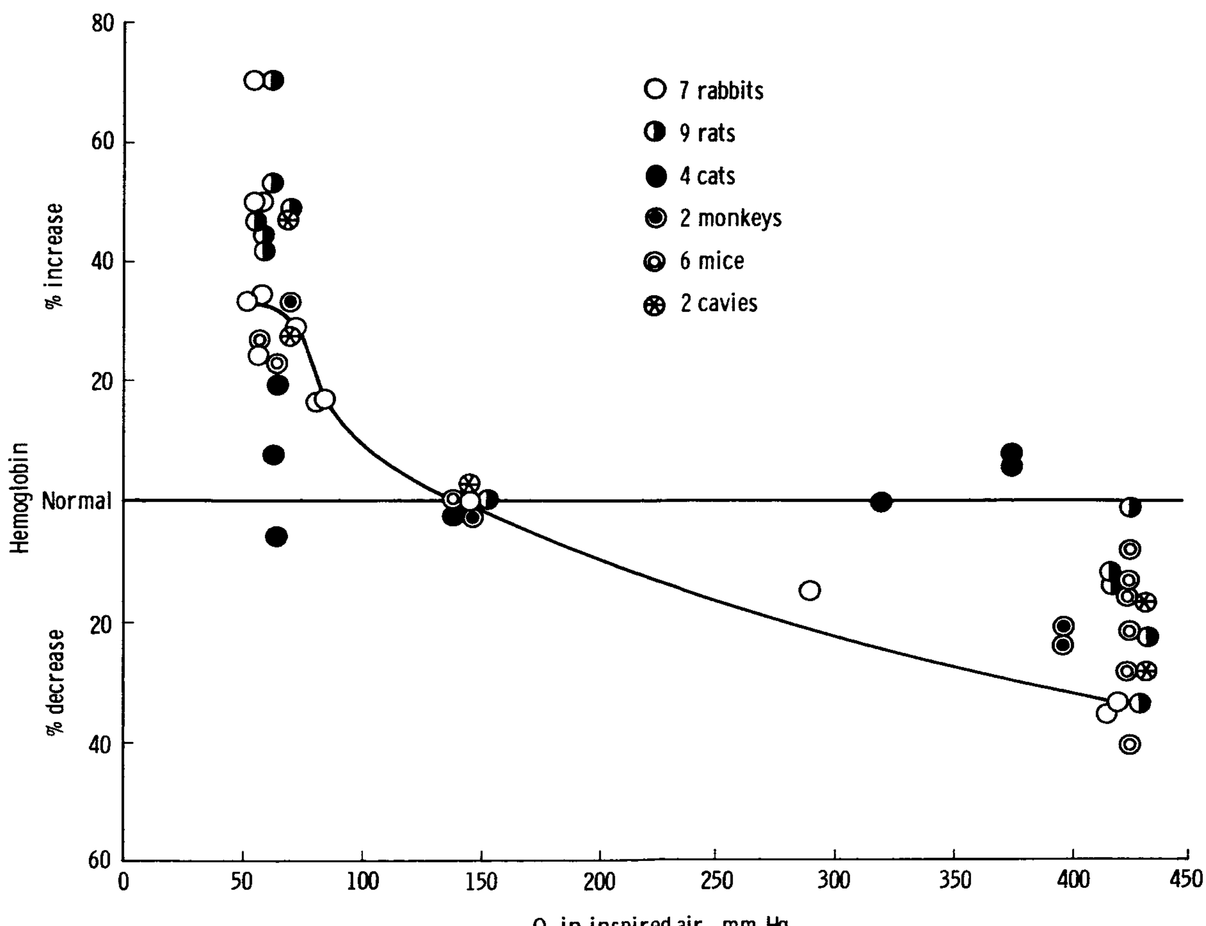

FIGURE 4.—*Relation between hemoglobin and oxygen pressure in the inspired air during prolonged exposures. The curve is drawn through points taken from one rabbit.* (AFTER CAMPBELL.[24])

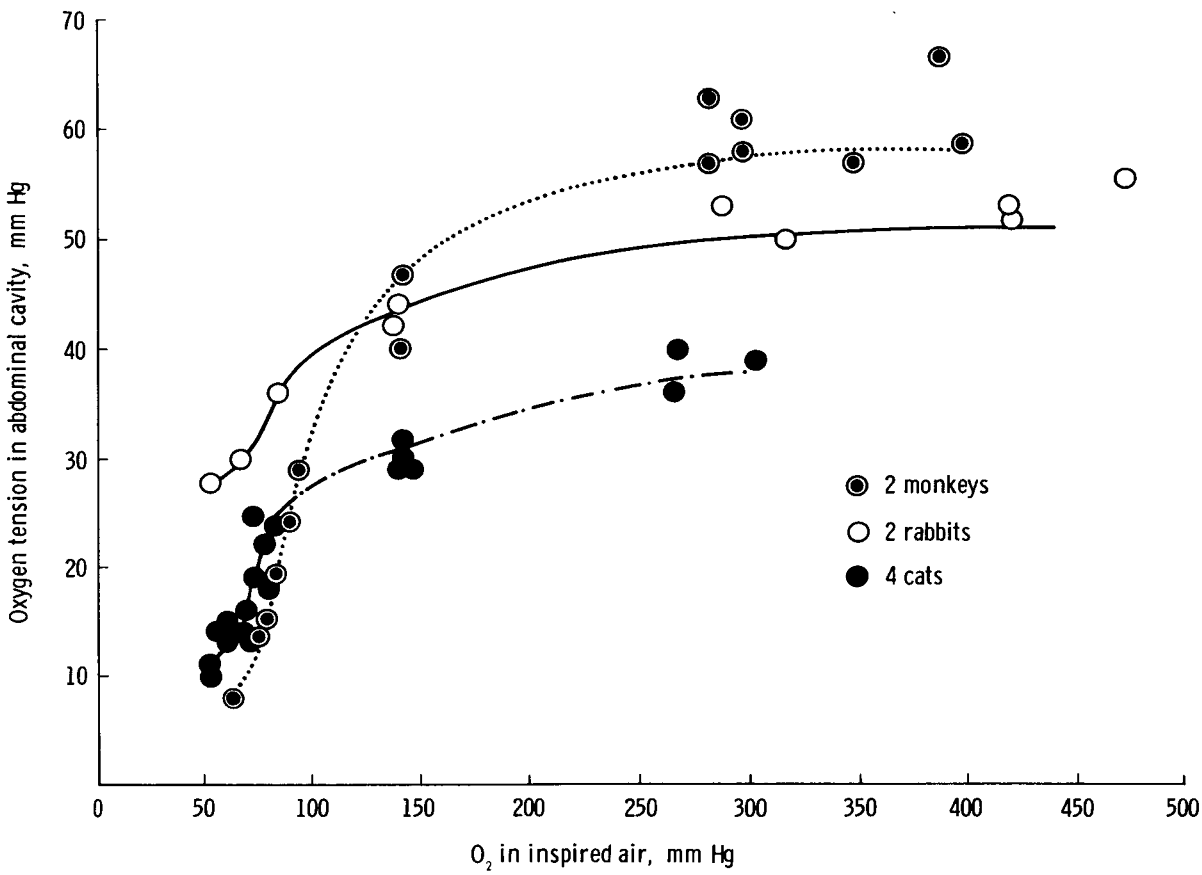

FIGURE 5.—*Relation between oxygen tensions in the abdominal cavity and oxygen pressure in the inspired air during prolonged exposures.* (AFTER CAMPBELL.[24])

providing a normal inspired oxygen tension. The carbon dioxide and nitrogen did not exceed 5 mm Hg for either gas. Under these conditions, animals appeared normal in most respects related to behavior, growth, and reproduction. In several cases, however, animals died of atelectasis within 48 hours of being placed in the chamber. Since it is more of a problem of nitrogen lack than oxygen excess, this atelectasis problem is discussed in Chapter 4.

Cook and Leon [36] have recently studied the threshold levels of p_{O_2} required for toxic effects in mice and male squirrel monkeys with temperature controlled at $25 \pm 5°$ C and relative humidity in the 87 to 91 percent range. Table 1 presents the results obtained with groups of 20 mice at each partial pressure. No purity analysis of the oxygen is reported. It would appear that the 570 to 646 mm Hg range is the threshold for mice. These results are similar to those in the older literature. Hemosiderosis was noted in the spleens of many of the animals, suggesting a hemolytic process; however, a mention that one of the controls showed the same defect tends to invalidate a hypothesis that the spleen pathology was of primarily hyperoxemic origin. Above 624 mm Hg, the classical lung pathology of OHP was found. The mice exposed to oxygen tensions of 358 and 548 mm Hg showed only occasional thickening of the alveolar membrane.

Several interesting new findings mark the autopsies of the mice exposed at 548 mm Hg. The first death occurred at 336 hours and the next one at 1,481 hours. The early death was discounted as due to "other factors." The other animals of this group demonstrated a progressive spastic type of paralysis starting at the 57th day. No damage to the anterior

TABLE 1.—*Lethality of Oxygen for Male C–57 Mice* [AFTER COOK AND LEON [36]]

Absolute pressure, mm Hg	Oxygen tension, mm Hg (a)	Average initial wt., gm	Time of first death, hr	LT_{50}, hr (b)	Number of spleens showing hemosiderosis
1, 140	1, 118	21. 8	25. 5	29. 25	3
760	738	24. 2	68	91. 5	4
646	624	26. 0	115	151	2
570	548	22. 5	[c] 1, 481	2, 448	1
380	358	21. 6	No deaths; experiment terminated at 28 days		2

[a] The difference between absolute pressure and oxygen tension represents water vapor pressure, which was found to be 22 mm Hg (90% humidity at a chamber temperature of 25° C).
[b] LT_{50}=lethal time for 50% of the animals.
[c] One death occurred at 336 hr due to other factors.

horn cells was noted and the "cortico-spinal tracts" were invoked as site of the primary defect. The livers were icteric in 50 percent of the animals and showed "fatty degeneration." The authors suggest that chronic oxygen at these relatively low tensions causes a "preferential poisoning of specific enzymic systems which results in symptoms similar to those produced by avitaminosis." Another possibility they mention is that "specific dietary elements are inactivated or in some manner made useless. This leads to death, not by anoxia due to lung damage, but probably by toxemia due to the reduction in the capacity of the liver to detoxify."

These authors quote Gerschman [63] as stating there is a difference in pattern of death of mice exposed to oxygen at pressures above and below 624 mm Hg. A review of Gerschman's paper does not reveal the differences that Cook and Leon report. Gerschman appears to be separating pulmonary deaths at relatively low oxygen tensions from convulsive deaths at oxygen tensions above 1 atmosphere. Cook and Leon are referring to pulmonary death at less than 1 atmosphere as compared with hepatotoxic and paralytic death at even lower tensions.

The conclusions of Cook and Leon are open to question. The mice were reportedly abnormal in that one control had splenic hemosiderosis. One might even interpret the hepatotoxic and paralytic death as due to the activation of a latent hepatotropic and/or neurotropic virus. That latent viruses can be activated by ionizing radiation is well known to bacteriophage geneticists who routinely use this technique for converting temperate bacteriophage to lytic types.[94] Since the mechanisms of action of high oxygen tensions and ionizing irradiation appear to be similar, oxygen may be expected to have this capacity. There is one Russian reference on the effects of OHP on neurotropic viruses,[124] but it was not available for review.

Cook and Leon also reported in this paper that two squirrel monkeys survived in good health for 80 days (termination of experiment) at an oxygen tension of 546 mm Hg; and two died at 622 mm Hg, at 367 and 377 hours. "Moderate lung damage was found." These monkeys, however, were out of the chamber for at least 1 hour a day. This fact suggests that the survival-time figures are higher than would be expected from a well-controlled experiment. Penrod [131] reported that intermittent exposure to nitrogen has a palliative effect on lung pathology, especially where an atelectatic tendency is present.

Throughout this study no mention is made of testing for leakage of room air into the apparatus or even of sampling the chamber for oxygen and carbon dioxide concentrations. Inadequate controls were reported in parts of this paper. The interpretations of Cook and Leon regarding the mechanism of death in mice at low oxygen tensions can be accepted, therefore, only with the strongest reservation.

The studies of Edwin Hiatt [89] of Ohio State University revealed some interesting results on work under contract with the U.S. Navy. Results in the first group of rats, which reportedly failed to grow in "100 percent" oxygen environments at 33,000 feet with 1 percent N_2 and CO_2 contamination, proved to be invalid. Apparently the total-pressure control was poor and the animals were hypoxic much of the time. The carbon dioxide and water vapor of the lung were reportedly not considered in the choice of altitude. An experiment with a second batch of rats was, therefore, run under conditions similar to those of MacHattie and Rahn [108] at a steady pressure of 190 mm Hg with 1 percent N_2 and CO_2 contamination. These animals showed no symptoms in a 24-day run. No atelectasis was demonstrated; no change in growth rate or feed consumption of these adolescent animals was noted. There was no difference in final hemoglobin levels between the experimental and control groups.

Berry and Smythe [18] have recently presented results on the latest experiments with mice kept for 3 to 4 weeks at simulated altitudes of 30,000 and 34,000 feet.

They used 100 percent "medical" oxygen; no mention is made of gas sampling or leakage control. Control mice were placed at 14,000- and 20,000-foot altitudes in air (447 and 349 mm Hg). The experimental situation was impaired by removing the animals for "a few minutes each day required for removal of bedding and provision of fresh food and water." No mention was made of carbon dioxide or humidity control.

The animals at both 30,000 feet ($p_{O_2}=226$ mm Hg) and 34,000 feet ($p_{O_2}=187$ mm Hg) fared well. They were metabolically normal except for an increased urinary nitrogen excretion. They were able to maintain an equivalence of 90 percent or more between increase in total carbohydrate and decrease in total body protein after cortisone injection under fasting conditions. This was interpreted as a demonstration of unaltered ability to carry out gluconeogenesis from non-nitrogenous compounds made available by protein catabolism.

Exact cause of the nitrogen loss is as yet not evident, but it is reported that the anoxic controls at 20,000 feet demonstrated the same nitrogen loss. Oxygen toxicity is probably not at fault. These investigators suggest that either "lowered barometric pressure itself or the conditions in the chambers" are responsible for the large resting level of urinary nitrogen. This nitrogen loss did not appear to be serious. Over a 17-hour fasting period sea-level controls lost 13.3 ± 4.7 mg urinary nitrogen per mouse, the 30,000-foot mice lost 20.7 ± 4.7 mg, and the 34,000-foot mice lost 21.1 ± 4.4 mg. It will be worthwhile to follow up these nitrogen abnormalities.

CHAPTER 3

Effects of High Oxygen Tension in Humans

THERE HAVE BEEN many studies of oxygen toxicity in humans at pressures of less than 1 atmosphere. In most of these, control of the oxygen tension was poor due to use of tents or masks for short or intermittent periods, and such experiments will be discarded as having no validity in this study. The experiments will be described individually. An attempt will be made to present findings chronologically and evaluate the frequency and circumstance of the pathological findings. The earlier reviews [8, 151] do not do this and suffer much for it.

TENSIONS OF 0.4 TO 1 ATMOSPHERE

Richards and Barach Experiments

Richards and Barach[139] reported that two men living in a well controlled environment of 45 percent oxygen at 1 atmosphere pressure (p_{O_2}=343 mm Hg) in chambers and tents for 1 week had no symptons. A slight increase in carbon dioxide tension in the blood was noted. Barach [6] also made reference to data from a psychiatric ward which suggests that at an oxygen concentration of 50 percent at 1 atmosphere (p_{O_2}=380 mm Hg), men showed no symptoms of oxygen toxicity for 2½ months.[90]

Clamann and Becker-Freyseng Experiments

During their self-experiment in 1939, Clamann and Becker-Freyseng [30] remained for 65 hours continuously in 0.9 atmosphere (578 mm Hg) of oxygen in a 40-cubic-meter chamber. The relative humidity was 67 percent and carbon dioxide 0.3 to 0.8 percent, with temperature at 19° to 21° C. During the first day, there was no discomfort. During the second day, Becker-Freyseng had decreased vital capacity and Clamann had median nerve paresthesia. During the third day, Becker-Freyseng had median nerve paresthesia and both had paresthesia in the toes. The vital capacity of Becker-Freyseng was lowered by 30 percent and his pulse and temperature were elevated; EKG and chest examination were normal. Clamann had a bout of tachycardia. On the fourth day, Becker-Freyseng awoke, felt sick, and vomited mucus. The experiment was terminated. Both men felt fatigued; EKG's were normal. Clamann's vital capacity was reduced by only 20 ml. Becker-Freyseng was oversensitive to noise and light and was diagnosed as having bronchitis. Fever, vital-capacity defect, and paresthesia lasted several days.

Examination after 24 and 48 hours in 0.9 atmosphere oxygen revealed no change in the erythrocyte count, but hemoglobin fell from 17.3 gm to 16.2 gm on day 2, and returned to 17.2 gm on day 3. In both subjects, leukocytes rose to 12,000 per ml.

Becker-Freyseng and Clamann [11, 12] breathed 82 to 90 percent oxygen for 65 to 72 hours at a simulated altitude of 29,520 feet. Oxygen pressure was in the range of 190 mm Hg to 210 mm Hg. No untoward symptoms were reported. These investigators concluded, on the basis of their experiments and others, that oxygen at a partial pressure of no more than 425 mm Hg was probably harmless for long periods of time. At any altitude above 12,300 feet, 100 percent oxygen "appeared safe" to these investigators. Figure 6 is Mullinax and Beischer's [117] transposition into English units of the curve from Becker-Freyseng and Clamann.[11, 12]

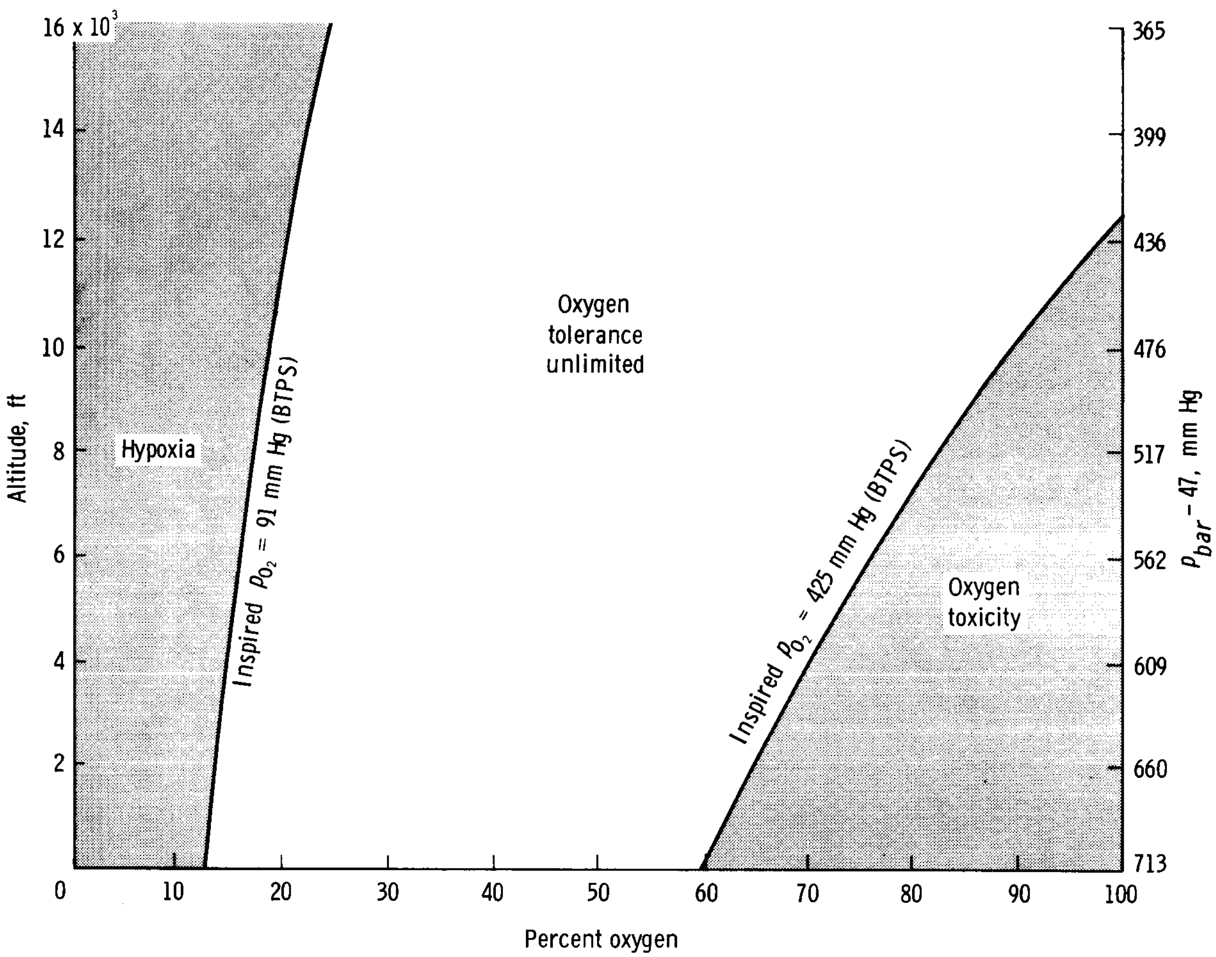

FIGURE 6.—*Oxygen tolerance in man.* (AFTER MULLINAX AND BEISCHER.[117])

Comroe et al. Experiment

Comroe et al.[35] had 34 healthy young men breathe oxygen without intermission for 24 hours, some of them in Heli-ox closed-circuit rebreathing devices and others with U.S. military oxygen masks. In relating the symptoms, the authors did not distinguish between these two groups. The oxygen masks were uncomfortable for the subjects. Twenty-eight out of 34 subjects who breathed 1 atmosphere oxygen felt substernal pain, as did 5 of 9 subjects on 0.75 atmosphere oxygen (570 mm Hg) with 15-minute intervals on air every third hour. None of the 10 subjects who breathed continuously 0.5 atmosphere oxygen (380 mm Hg) or the control subjects on air reported this pain. Pain became more intense during the test, with times of onset from 14 to 22 hours. It was intensified by coughing and deep breathing. In all but three subjects, pain disappeared within 12 hours. In three subjects, it lasted 16, 24, and 31 hours after exposure. Comroe et al. attribute these symptoms to "tracheo bronchitis." (Behnke et al.[14] reported the same findings in a subject breathing 1 atmosphere oxygen for 4 hours.)

About 40 or 45 percent of those breathing 100 percent oxygen had nasal congestion or coryza; 25 percent had ear trouble. The dry oxygen caused sore throats in 30 percent of the subjects and intermittent cough in 50 percent; 20 percent of dry-air controls were so afflicted. Fatigue was noted in 25 percent of subjects on pure oxygen and in 10 percent of controls on air. Five subjects had joint discomfort; three had paresthesia; three had palpitation; and seven felt giddy or had headache.

Comroe found a reduction in the vital capacity of 63 of the 80 persons who had breathed 0.5, 0.75, and 1.0 atmosphere oxygen for 24 hours, but no lung changes could be detected on auscultation or X-ray. Recognizing that the symptoms might have been due to low nitrogen as well as excess oxygen, Comroe et al. also placed six men at 18,000-foot altitude on 100 percent oxygen (380 mm Hg) by mask for 24 hours and found no symptoms.

Ohlsson Experiment

In 1947 Ohlsson [122] studied men in a chamber containing 78 to 88 percent oxygen at 1 atmosphere pressure (594 to 670 mm Hg) and 1.0 to 2.7 percent carbon dioxide for 53 to 57 hours; and two men during a control exposure to 21 to 35 percent oxygen and 0.4 to 2.0 percent carbon dioxide. The chambers had variable oxygen and carbon dioxide in the above ranges due to poor control techniques. The relative humidity was between 45 percent and 72 percent and the temperature was between 16° and 20° C.

Three of the six subjects breathing oxygen complained of nasal obstruction and ear blockage; X-rays showed sinus opacities and fluid was seen in the ears. Within 24 hours, four of the six complained of substernal distress "like a stitch"; this was intensified by coughing and deep breathing. Headache, anorexia, nausea, and giddiness were reported by four of the six, and paresthesia lasting 24 hours after the experiment was recorded in one of the subjects. The men were aware of the danger of oxygen toxicity; yet, control subjects experienced none of these subjective sensations, even though unaware of their gaseous environment. After 48 hours in oxygen, the men complained of generalized unrest, absentmindedness, and incapacity for concentration with a tendency to be distracted and show cyclothymic behavior patterns. The termination of this experiment was actually brought about by concern regarding these mental aberrations.

An acceleration of respiratory rate was seen in two of the oxygen subjects. After a few hours, vital capacity decreased in five of the six subjects, and when experiments were discontinued the vital capacities had fallen 400 to 1,600 ml below control values. After the exposure, it took 3 days for vital capacity to return to normal. During this period subjects complained of fatigue, breathlessness, and exhaustion on slight exercise. One subject had bubbling rales at the left lung base. No X-ray changes were seen within 24 hours after exposure. There were no changes in the formed blood elements except for a slight rise in the white blood count from 7,600 to 12,000 cells per ml in one subject. No changes in "alkali reserve," chloride, or total base content were noted. There was a slight rise in pulse rate of two subjects. Blood pressure and EKG's were all normal. There was no statistically significant change in basal metabolic rates noted.

TENSIONS OF 0.2 TO 0.4 ATMOSPHERE

Hall and Martin Experiment

More recent studies start with the demonstration by Hall and Martin [82] that a subject could tolerate 3.5 psi at 100 percent oxygen (181 mm Hg) in a Navy full-pressure suit for 72 hours without symptoms other than a pustular dermatitis and eye, nose, and throat irritation. The dermatitis was postulated to be due to unsanitary skin conditions, irritation, and dry oxygen. Pulmonary function tests were normal, as were hematological studies, urinalyses, and blood chemistries, except for an eosinophil and 17-ketosteroid "response to stress."

Hall and Kelly Experiment

In a similar study, Hall and Kelly [81] exposed two men, one in a pressure suit, to 3.5 psi and 100 percent oxygen (181 mm Hg) for 5 days. There was no significant change in vital capacity or laboratory findings. Irritation of eyes, nose, and throat was again reported. The report was not available in time for an evaluation of the details.

Michel et al. Experiment

In 1958 Michel et al.[114] at the Aircrew Equipment Laboratory, U.S. Naval Air Development Center, Philadelphia, placed six subjects in an altitude chamber at 10,000 feet (523 mm Hg) and exposed them to 80 percent oxygen (418 mm Hg), equivalent to 55 percent oxygen at 1 atmosphere, for 168 hours (7 days). The main symptom reported by the subjects was substernal tightness on deep inspiration from the second day to the end of the study on the seventh day. Middle-ear blockage was recorded by all. Dermatitis was reported and thought to be caused by fire-retarding chemicals in flight suits. Pulse and respiration were normal. Two subjects showed a decrease in vital capacity; and one, in an X-ray, showed an area of probable atelectasis that disappeared 24 hours after the examination. All symptoms disappeared within 24 hours after the conclusion of the experiment. Hemograms were all within normal limits, as were urinalyses.

Roth-Gaume and Steinkamp et al. Series

In 1956 Roth and Gaume[142] initiated a series of experiments in the space-cabin simulator at the U.S. Air Force School of Aerospace Medicine, Randolph Air Force Base, Texas. The subjects were maintained up to 24 hours at 18,000- to 25,000-foot altitudes in 40 to 54 percent oxygen (equivalent to oxygen pressure of 150 mm Hg) with no apparent adverse effects. Even during periods when carbon dioxide rose in the chamber to 6 percent sea-level equivalent, a condition which should have potentiated toxic effects of oxygen, there were no specific pulmonary symptoms of oxygen toxicity.

Steinkamp et al.[152] continued these studies by conducting a 7-day experiment at cabin pressures of 380 mm Hg (18,000 feet) with oxygen in the 150 to 160 mm range. Fluctuations in oxygen up to peaks of 225 mm Hg were reported. Most of the time carbon dioxide tensions were kept below 4 or 5 mm Hg, though occasional peaks as high as 28 mm Hg were recorded. The relative humidity was in the 40 to 44 percent range. There were apparently no physiological effects attributed to oxygen toxicity in this unusual oxygen environment.

Welch et al. Experiments

In 1959 Welch et al.[166] kept subjects in the two-man space-cabin simulator at the U.S. Air Force School of Aerospace Medicine, Brooks Air Force Base, Texas, for 30 days at 18,000 feet (7.3 psi) with oxygen enriched to 40 percent (p_{O_2}=150 mm Hg) and for 17 days at 33,500 feet (3.7 psi) at 100 percent oxygen (p_{O_2}=176 mm Hg). In preliminary studies with animals, the latter condition caused no apparent pathological changes.

In the experiments with humans, there were no respiratory symptoms during the 18,000-foot run (p_{O_2}=150 mm Hg), but there were some interesting findings at 33,500 feet with 100 percent oxygen. As soon as altitude was reached, complaints were voiced of dryness of the respiratory tract, nasal congestion, and eye irritation. These were probably due to the dryness of the gas since symptoms decreased as relative humidity levels rose at 24 hours; symptoms disappeared at 72 hours. Minimal paresthesia was noted in calves and arms. Aural atelectasis required clearing of the ears every 2 hours.

On the ninth day of exposure, one subject noted mild retrosternal pain which increased on inspiration. This pain continued with increasing severity for 24 hours. An increase in pressure by addition of oxygen to p_{O_2}=244 mm Hg relieved this distress and the subject was asymptomatic thereafter. Since in all the previous human studies (discussed above) using higher oxygen tensions no relief of symptoms occurred until the p_{O_2} was dropped, the symptoms in this experiment were probably not caused by high oxygen tension *per se*. Atelectasis is the only explanation.

Pulmonary function studies were of interest in that a reduction was noted in vital capacity, which reached a minimum of −10 percent in 5 days and remained at this level for the remainder of the experiment. (Becker-Freyseng and Clamann [12] reported that in a 3-day experiment at 30,000 feet with 100 percent oxygen (p_{O_2}=225 mm Hg and 4.36 psi) the vital capacity dropped suddenly to −20 percent on the first day and returned to more normal limits thereafter.) It is of interest that Rahn and Hammond [137] report a similar though less severe drop at 14,200 feet and 20 percent oxygen. No adequate explanation is available for the vital-capacity decrease observed by Welch, though the Rahn study suggests that oxygen *per se* is possibly not at fault. Postflight X-rays revealed no atelectasis, and timed vital-capacity studies revealed no improvement on the second of paired tests as would be expected if collapsed alveoli had been opened on the first test.

Temperature, pulse, and respiration were normal. There was, however, a significant decrease in diastolic pressure in several of the subjects. Welch also reported decreases in the "treadmill time of Balke"[5] and excessive pulse rate after work. These decrements were somewhat larger than, but in the same range as, those produced by 4 weeks of bed rest. Orthostatic tolerance on the tilt table was not greatly changed. The myocardial (EKG) changes of prolonged PR interval and of atrial and ventricular premature beats were seen on the tilt table, Master's test, and Valsalva maneuver. These were more marked than those seen on bed rest alone, but reverted to normal at the 2-month followup examination. Since both the 18,000- and 33,500-foot altitudes produced the same effect, oxygen is probably not the sole factor in these changes; nevertheless, it may contribute to these cardiovascular instabilities.

In both the 18,000-foot and 33,500-foot experiments, decreases in total body water, blood volume, and plasma volume were noted in significant excess of that expected from loss in body weight. In the 30-day run at lower altitude, the decreases were more severe. Fat deposition may partly account for this phenomenon. The recovery period was greater for the 17-day run than for the 30-day run. Adequacy of water supplies, relative humidity of 50 to 70 percent, and normal water clearance, urine flow, osmolar clearance, and free plasma osmolarity suggest that dehydration was not involved. Further experimentation is obviously necessary to account for these changes. The fact that they were more severe at 18,000 feet for 30 days than at 33,500 feet for 17 days suggests that oxygen is not a factor. A small decrement in psychological testing may be adequately explained by the experimental conditions other than the oxygen environment. Only aural atelectasis and possibly, though not probably, the substernal distress appear to be related to the increased percentage of oxygen in the environment.

In the above study of Welch, and, as a matter of fact, in almost all previous studies, the role of nitrogen has not been considered. It is suspected from evaluation of environmental conditions, apparatus, and oxygen sources that in the best "100 percent" oxygen conditions, only 90 to 95 percent was actually attained. Leaks into chambers at less than 1 atmosphere were probably the worst offenders. Sampling of the gaseous environment for percentage of inert components was hardly ever accomplished.

Helvey-Republic Aviation Corporation Experiment

A recent study of oxygen toxicity in sealed cabins was performed at Republic Aviation Corporation by Helvey.[87] The basic contribution of this study was an evaluation of oxygen toxicity in the "absence" of inert diluents. The material to be presented stems from a preliminary copy of the final report of this experiment.

Twenty-eight men were divided into 4 groups and placed for 14 days in a sealed chamber at sea level (control), 7.4 psi (380 mm Hg; 18,000 feet), 5 psi (258 mm Hg; 27,000 feet), and 3.8 psi (196 mm Hg; 33,000 feet). All three compartments of the chamber could be flushed with oxygen and so preserve the "diluent-free condition." The leak-rate design parameter was less than 0.01 ml STP dry air per second at 1 μ Hg pressure differential into 2,500 cu ft. Combustibles and "obviously" toxic materials were eliminated. The chamber was continuously flushed with 100 percent oxygen while vacuum pumps maintained the appropriate pressures. To keep down contaminants, there was a complete turnover of atmosphere once every 20 to 30 minutes.

About 600 to 700 liters of liquid oxygen per day were required. Two samples of this material were analyzed by the Air Reduction Company Laboratory, Murray Hill, N.J. (see table 2). The flow rate of oxygen was manually controlled. The "locking" of subjects and medical personnel into the chamber was initiated only after oxygen flushing to a maximum 0.5 percent nitrogen. This was combined with a 3-hour denitrogenation of the subjects. Nitrogen was measured with a Med-Science Electronics Nitroanalyzer and periodically with a gas chromatograph.

The oxygen was measured with a Chemtronics sensor. Intermittently, a Bendix time-of-flight

TABLE 2.—*Analysis of Liquid Oxygen* [AFTER HELVEY [87]]

Gas [a]	Quantity by volume	
	[b] 1	[c] 2
Oxygen	99.82%	99.82%.
Argon	0.1%	0.1%.
Nitrogen	0.01%	0.01%.
Total hydrocarbon such as CH_4.	17 ppm	16 ppm.
Methane	11 ppm	10 ppm.
Ethane	0.03 ppm	0.02 ppm.
Carbon dioxide	0.8 ppm	0.6 ppm.
Krypton	8 ppm	9 ppm.

[a] Other contaminants analyzed for but not detected: ethylene, acetylene, propane, propylene, i-butane and n-butane. Threshold of detection, 0.01 ppm by volume.

[b] Sample from gas outlet.

[c] Sample from liquid outlet, vaporized through copper coil.

mass spectrometer was used to monitor nitrogen, oxygen, carbon dioxide, and water, as well as to scan the mass range 0 to 100 for traces of other gases. During the experiments, carbon dioxide varied from 0.08 to 0.22 percent at sea level; 0.6 to 0.45 percent at 3.8 psi; 0.16 to 0.68 percent at 5 psi; and 0.28 to 0.55 percent at 7.4 psi. The relative humidity ranged through the experiments from 30 to 69 percent and the temperature ranged from 70° to 76° F. Intermittent Kitagawa analysis for NH_3, H_2, H_2S, and CO proved negative.

TOXIC MATERIALS

A review of the toxic hazards complicating the Helvey experiments reveals the following:

Tricresyl phosphate. Vacuum pumps were lubricated and sealed with tricresyl phosphate. If the chamber was constantly flushed and evacuated, this should have caused no trouble.

Lock foam insulation. The liquid-oxygen pumps in the chamber were insulated with freshly synthesized urethane polyether containing a plasticizer of 54 percent toluene and 46 percent diisocyanate with the final product having 27 percent toluene. Polyethers, as well as polyester foams, are sensitive to oxidation—the polyethers more so.[4] The companies manufacturing such foams were contacted for specific information on breakdown products under the experimental conditions.

Following a lead from the Elastomers Laboratory of E. I. du Pont de Nemours & Company, several references to the toxicity of these chemicals were obtained. Unfortunately, no work has been done on the breakdown products of this polymer in high-oxygen environments. However, the toxicology of toluene-diisocyanate (TDI) has been well studied.[60, 61, 138, 154, 177]

The paper of Zapp [177] includes a study of subacute exposure to TDI. In one experiment, ten 6-hour exposures to analytical concentrations of 1 to 2 ppm of TDI produced no injury to rats. In another experiment, one rat died after the eighth and one after the eleventh 6-hour exposure. Both showed emphysema, and one had definite bronchitis. After thirty 6-hour exposures, the other rats were sacrificed and microscopic signs of tracheobronchitis were noted. After seventy-nine 6-hour exposures to 1.5 ppm of TDI, four of five rats showed definite bronchitis when sacrificed.

Guinea pigs killed after twenty-three to seventy-nine 6-hour exposures all showed bronchitis and bronchopneumonia. One rabbit died after three exposures and one after five exposures to 1.5 ppm TDI. Both showed gastroenteritis as well as bronchitis. Other rabbits exposed for up to seventy-one 6-hour periods showed bronchitis and slight pulmonary edema.

No blood or urine studies are reported. These animal studies confirm earlier reports that TDI can sensitize humans to asthmatic bronchitis and cause direct irritation to the eyes and mucous membranes. As will be seen, none of Helvey's subjects demonstrated these symptoms. This, of course, does not rule out a sensitizing role of this compound in the Helvey experiments.

Though no studies of oxidation products of these plastics have been performed, Zapp has made several studies of the low-temperature pyrolysis products of the polymer. Exposure of rats to the products produced by a 6-hour 250° C pyrolysis of the elastomer foams polyurethane A, B, and C, neoprene, and rubber latex resulted in death from pulmonary edema and congestion. The neoprene and rubber polymers were more dangerous than the polyurethane. This author suggests that the maximum allowable concentration of TDI be set at 0.1 ppm in air. The lowest detectable level (smell) is 0.4 ppm and lowest symptomatic human level (burning of nose and throat) is 0.5 ppm in air.

In a recent study of high-temperature pyrolysis products of polyurethane foam,[107] it was shown that rats would die of bronchitis and pulmonary edema only when exposed to rather high concentrations of the material. Assuming that rats and humans are equally susceptible to the pyrolysis products, it would take 30 minutes of exposure to the products of a pound of plastic pyrolyzed into 250 cu ft of air to kill a man. It is impossible to extrapolate from these figures to the very slow oxidation product, if any, released in the Helvey study.

Helvey attempted an analysis of foams exposed in bell jars to 5 psi oxygen. There was no atypical Kitagawa color change in the toluene test and no benzene or toluene peaks were seen with the mass spectrometer. Flushing of the manned chamber should have eliminated toxic products, but it was reported that there were small unexplained peaks on the mass spectrometer records during the chamber experiments.

Mercury. During the course of the study, in the control and 3.8 psi runs six thermometers and one sling psychrometer were broken. After the control run, the stainless steel surfaces were "thoroughly" cleaned, expecially below the stainless steel floorboards. The chamber was continually exhausted from below the floorboards and heavy mercury vapor should have been swept out. The details of urinary-tract findings suggesting possible mercury poisoning will be discussed below.

General Medical Results

There was weight loss in all three experimental groups with a corresponding lack of interest in the ad lib food. The loss was most severe in the 3.8 psi group and least severe in the 5 psi group. The cause of this result is not clear and is complicated by other findings to be reported.

Sea-level run. Throat cultures from all subjects revealed β-hemolytic streptococci (β-strep), but no symptoms were reported. One subject had "an enlarged, tender spleen" on leaving the chamber, but remained well. The cause of this pathology is not apparent.

3.8 psi run. In spite of 3 hours' denitrogenation prior to the run, one subject developed, after 15 to 30 minutes, symptoms of neurocirculatory collapse and had to be removed. Two subjects had mild bends. It was felt that "chilling" during the "flush" period of denitrogenation in the lock "aggravated" the bends problem. On the third day, one subject began to cough during vital capacity examination. The cough cleared, but returned several days later in milder form. By the fourth day, all subjects had blocked ears which cleared with chewing gum. No X-ray signs of atelectasis were found. No symptoms of urinary-tract disorder were noted in two subjects who were later shown to have trace protein and hyaline casts in the urine.

5 psi run. Five of six subjects had head colds during this run and one had a mild sore throat. All subjects had, on occasion, a few colonies of β-strep in their throat cultures. One subject had a β-strep external otitis which was present before the run and responded to tetracycline, 250 mg by mouth for 5 days during the run. (The treatment of a single patient with a broad spectrum antibiotic certainly confuses the whole bacterial floral picture.) Conditions possibly related to oxygen and pressure were:

1. Eye irritation in four of six subjects on the second to sixth day.
2. Mild substernal discomfort or "clogged" chest in two subjects. This increased with cough or deep inspiration.
3. Aerotitis in all subjects, with some hearing loss, especially during the periods of head colds. Adrenalin nose drops, chewing gum, and nocturnal arousal for ear clearing seemed effective.

7.4 psi run. No substernal distress was reported. Mild aerotitis was seen in several subjects. One subject (who had β-strep cultured from throat) coughed up a teaspoonful of red blood on the ninth day. This was the only such occurrence. Several fecal specimens of other subjects were checked out for occult blood during the experimental run and found to be positive. Postrun followups were negative.

LABORATORY FINDINGS

Cardiopulmonary. The arterial p_{O_2}, p_{CO_2}, and pH were normal in all subjects. In the 7.4 psi run (380 mm Hg) the mean oxygen content on the fifth day at altitude was 20.20 vol% (14.1 gm% hemoglobin) as compared with 17.0 vol% (14.38 gm% hemoglobin) on the postrun day. These results, along with the fact that p_{O_2} values were normal (266 mm Hg) and that oxygen capacities were (day 5) 19.3 and (day 14) 18.1 vol% rather than the 19.3 and 19.2 vol% expected, suggest an abnormal pigment in the blood, i.e., methemoglobin. "The conversion of this pigment to oxyhemoglobin during the equilibration in room air" could explain why capacity measurement was higher than content measurement. The greater inherent error of the Natelson microgasometer method at high saturation or errors involved in the relative slowness of the method when dealing with many samples may have contributed to the lower measure of saturation by the Natelson microgasometer. If 5 percent of hemoglobin were oxidized to methemoglobin, the 5 percent discrepancy in saturation could be accounted for on a basis other than that of experimental inaccuracies.

There were no changes in static or timed vital capacity. The maximum breathing capacity showed an immediate increase during the altitude runs, probably as a result of decreased gas density. Marked coughing following these tests may represent a significant borderline atelectatic condition. There was an unexplained decrease in maximum breathing capacity at the end of the 3.8 psi run. Total lung capacities and diffusion capacities were normal.

Biochemistry. Blood electrolytes, glucose, and blood urea nitrogen were normal. The high 17-hydroxsteroids of the control run may reflect the stress involved in being the first group. In the experimental run, only at the end of the 5 psi run did elevated levels appear.

Microbiological. There was no change in flora of the throat. Fecal floras of each group changed, tending to become similar in each group by the end of the run. There were several unusual types of anaerobes in the fecal floras. Strict anaerobes predominated over aerobes "by a factor of 1000" and were the predominant flora in 20 of 23 subjects. Variations between the four groups could be explained by individual variables and do not appear to be significant. There was a tendency toward a greater percentage of skin aerobes at the end of the run.

It would appear that while the fecal environment remains anaerobic enough for normal anaerobe growth, the skin conditions are such as to select against the strict anaerobes. This is as would be expected.

Urinalysis. During the runs, occasional traces of protein and casts were seen in all but the last (3.8 psi) run. When many casts, as well as protein, were found in all 3.8 psi subjects (two thermometers and a psychrometer broke during this run), followup urines were obtained in other groups. Protein and casts of waxy and granular types were found more frequently during followup than during the runs and persisted 2½ months in the 5 psi group. At 3 months the urines appeared more normal.

These findings are consistent with renal damage. The question of mercury poisoning rears its head. Unfortunately, the fact that no mercury was found in the urine after "qualitative" tests does not rule out mercury poisoning. Neal and Jones [120] in a review of chronic mercury poisoning of workers in the hat industry point out that a quantitative spectrographic technique revealed mercury in urine specimens of only 3 out of 10 patients (all of whom had severe chronic symptoms of mercury poisoning). There is apparently no correlation between degree of symptoms and urine mercury levels. Less than 23 percent of the patients had stippled red blood cells. Studies of subacute exposures to mercury vapor or fumes are rare, especially those in which no clinical symptoms are present in the face of abnormal urine. Areas of acute exposure in men welding metal seals have been reported,[169] but the journal was unavailable for review. The figure of 0.25 mg of mercury per liter of urine as an indication of chronic mercury poisoning, which Helvey quoted, may be high. Neal et al.[119] found an average of only 0.017 mg per liter in urine of men exposed to 0.05 mg of mercury per cubic meter of air. (The maximum allowable concentration is 0.1 mg/m^3.) Exposure to 0.25 mg/m^3 gave an average of 0.29 mg per liter of urine with a range of 0 to 1.1 mg per liter. These were the probable exposure concentrations in this experiment. Helvey reports his tests were "qualitative." It has been suggested that some of the urine he has saved be submitted to a good state toxicology laboratory for spectrographic analysis. It must be remembered, however, that normal people excrete about 0.05 micrograms of mercury per day in the urine.[153]

One must also keep in mind that high oxygen or low nitrogen conditions may combine with subthreshold mercury intoxication to give kidney damage. Both mercury and oxygen can inactivate —SH groups of the cells. No studies of this potential synergism could be found. Lack of correlation between p_{O_2} and urinary pathology does not rule out high p_{O_2} as the sole factor since individual variations may predominate at threshold levels of toxicity. Low nitrogen may, of course, explain the uniqueness of these findings. The previous discussion of the actual conditions of this mercury exposure would suggest the mercury factor to be on the low side of probability as a cause of urine changes.

These subjects should have frequent repeated urinary-function studies. This should be done for the patient's sake as well as for biological baselines in case the urinary findings are typical of the high p_{O_2}, low nitrogen environments.

Hematology. The hematological findings are the most striking of all. Helvey's summary appears adequate for this discussion:

1. The hematological system of the sea level group showed no significant change, except a reversal of the white blood cell polymorphonuclear and lymphatic ratio.

2. The 5.0 psi group (except Subject 35) demonstrated a slight anemia, microcytosis, increased osmotic fragility, and minimal erythroid hyperactivity. Subject 37 had a loss of over 2.0 gm% hemoglobin and a 2.2% reticulocyte count. The follow up examinations nine and eleven weeks post-run more clearly demonstrated that the hematological abnormalities of the subjects had persisted. The Price-Jones curve continued to show flattening and broadening of the base with a concomitant microcytosis. The morphology of the red blood cells showed the following abnormalities: anisocytosis, spherocytosis, abnormal distribution of hemoglobin stippled cells, polychromasia, normoblasts, Howell-Jolly bodies and Cabot's ring cells. Additionally, there was a 2.1 gm% decrease in mean hemoglobin nine weeks post-run, followed by a 0.8 gm% increase in hemoglobin concentration with a 3% reticulocyte response eleven weeks post-run. Subject 35 (later shown to have thalassemia trait) demonstrated a hemolytic anemia with a progressive decrease in hemoglobin from 15.8 to 10.5 gm%. Post-run examinations indicate that his blood picture appears to have stabilized between 12 to 13 gm% hemoglobin with a continued abnormal morphological picture consistent with his hereditary hemoglobin defect (thalassemia trait).

3. The 7.4 psi group exhibited a fall (2 to 3 gm%) in hemoglobin concentration during the first 48 hours, with a rise in bilirubin and urine urobilinogen levels. Reticulocytosis occurred on the 3rd day and persisted at 3.0 to 5.5%. Normoblasts, macronormoblasts, and macrocytosis appeared, indicating increased erythropoiesis. The latter was also noted in the post-run bone marrow examinations. In the white blood cells of the peripheral blood there was a marked degree of vacuolization of the cytoplasm and nucleus and an occasional young white cell was seen toward the end of the experimental run. After the fourth day, the hemoglobin concentration leveled off except for a mean one gram drop on the eleventh day. Thereafter, the hemoglobin level rose and the reticulocytosis decreased. In the eight weeks post-run examination there was a slight decrease in hemoglobin and a continuing reticulocytosis, macrocytosis, and there were morphological changes in the red and white blood cells, suggesting the persistence of the hematological abnormalities.

4. The hematological picture of the 3.8 psi run subjects resembled that of the 5.0 psi run subjects, except for a more marked reticulocytosis. The Price-Jones curve showed marked flattening and broadening with microcytosis. Morphological changes included normoblasts, spherocytes, and microcytosis, and anisocytosis of the red blood cells. Follow up examinations three and five weeks post-run on three available subjects show a continuation of the hematological abnormalities present during the experimental run. All subjects after two weeks of exposure to 100% oxygen atmospheres at reduced pressures exhibited some hematological abnormalities which have persisted. These alterations generally suggest erythroid hyperplasia secondary to hemolytic processes.

These findings are all rather puzzling as far as etiology is concerned. The toxic factors invoked to explain the urinary findings still becloud the issue. High p_{O_2} appears in this case to be the most probable initiating or aggravating factor. As was mentioned previously in the discussion of mechanisms of oxygen toxicity, individual variations should play a great role at the threshold p_{O_2} tensions obviously present in these experiments. As was pointed out in the animal studies of Campbell,[25] a tendency toward a "hemolytic" anemia (excess prussian-blue staining material in spleens) is seen in animals under similar p_{O_2} conditions. The animal blood studies were, of course, not as sophisticated as these. They showed little reticulocyte response to lowered hemoglobin. The studies of Hiatt [89] suggest that the threshold of hemoglobin effect in mice lies between p_{O_2} values of 190 and 420 mm Hg.

None of the previous human studies discussed above showed significant changes in the red cell picture even at high oxygen tension. There is, however, a report in the literature by Tinsley et al.[160] in which normal humans and patients with sickle cell, congenital hemolytic, and pernicious anemia were given from 50 to 100 percent oxygen by mask at 1 atmosphere. The mask discipline was reportedly poor. In the normal subjects, small but significant decreases in red blood cells and hemoglobin were noted during the first few days of the experiment and persisted until the oxygen was removed. Reticulocytes fell off by one-third and radioactive iron uptake was reduced during oxygen administration. The reticulocyte response of pernicious anemia patients to liver extract was reduced in magnitude and duration by 70 percent oxygen and resumed to a peak 10 days after cessation of oxygen. Changes were more dramatic than in normal subjects.

One may speculate, of course, that either erythropoietin level or the marrow response to normal erythropoietin is reduced by the elevation in marrow p_{O_2} levels. Jacobson et al.[95] have reported that any increase in supply of oxygen when the demand remains normal (such as through transfusion and polycythemia) produces in the rat a "profound decrease in erythropoiesis" which is relieved by addition of anemic plasma rich in erythropoietin. The kidney has been shown to be the site of formation of erythropoietin in rats. That the kidney and not the local marrow p_{O_2} appears to control in humans the level of erythropoiesis can be deduced from a case of a patient [171] with patent ductus distal to the subclavian artery. The normally oxygenated sternal marrow showed erythroid hyperplasia along with the hypoxic marrow below the ductus. Indeed, humans with different types of renal disorders are known to become polycythemic.[164] One might speculate then that hyperoxic stimulus in the kidney may be responsible for the decreased erythropoiesis reported by Tinsley et al.[160]

It appears pertinent at this point to review the relationship between oxygen toxicity and the mode of action of erythropoietin. Cobalt has been known for many years to stimulate polycythemia. A recent paper by Linkenheimer [105] indicates that cobalt feeding stimulates polycythemia in rats and this response is augmented by erythropoietin. The action of cobalt in catalyzing the breakdown of peroxides [42, 63, 71] was discussed in Chapter 1. It may well be that the level of red blood cells in the body is actually determined by the peroxide concentration within specific kidney cells. Hemorrhage, anemia, and hypoxia decrease peroxides by lowering the intracellular concentration of oxygen. Cobalt accomplishes this directly by breaking up the peroxides and eliminating the ultimate chemical stimulus in these cells for the elaboration of erythropoietin. It may be that the reticulocyte response seen in Helvey's experiment was actually supressed by this indirect effect of oxygen on marrow activity.

In view of positive reticulocyte response and signs of hemolysis in the Helvey experiment, a review of the literature on the exposure of red blood cells to oxidative environments is in order. As was discussed in Chapter 1, deprival of vitamin E [65, 110, 157] leads to a syndrome reminiscent of oxygen poisoning. Indeed, vitamin E deficiency caused the hemolysis of red blood cells in rats exposed to an oxygen partial pressure of 5 atmospheres for up to 200 days. Only the vitamin E deficient animals showed *in vivo* hemolysis and severe red cell fragility *in vitro*. No description was given of the red cells. Vitamin E deficiency alone makes red cells of rats sensitive to hemolysis in the dialuric acid hemolysis test. Tocopherol (a vitamin E) or the reducing agent methylene blue given to these rats before exposure to 5 atmospheres of oxygen prevented hemolysis.

Rose and György[140] demonstrated that catalase will inhibit the dialuric hemolysis test. Hydrogen peroxide at low concentration only slowly hemolyzes vitamin E deficient cells. It was shown that many organic antioxidants inhibit the dialuric acid hemolysis. Hydrogen peroxide was suspected of affecting the dialuric test and evidence was presented that hydrogen peroxide may be an intermediate to the actual intracellular hemolytic agent. In no case have erythrocytes of humans or tocopherol-treated rats been hemolyzed by dialuric acid alone. The low concentrations of peroxide used in these studies will hemolyze only 5 percent of human red blood, though an occasional human value up to 40 percent was noted. It thus appears that under the proper sensitizing conditions, oxygen at high pressures may bring about hemolysis of the red blood cells of rats, possibly through a peroxide mechanism.

Experience with human red blood cells indicates that under the proper sensitizing conditions, they too are susceptible to oxidative degeneration. As Jandl et al.[96] have discussed, hemolysis due to primaquine sensitivity appears to be related to a hereditary reduction in glucose-6-phosphate dehydrogenase in red blood cells. This enzyme is required for the generation of reduced triphosphopyridine nucleotide via the pentose shunt pathway. Other reducing substances such as glutathione are thus regenerated. This oxidative pathway appears to be deficient in aging cells and the resulting antioxidant deficiency is probably ultimately responsible for their eventual hemolysis.

As was discussed in the section on molecular mechanisms, the enzymes of the glycolytic pathway which generates reducing agents appear to be the ones sensitive to oxygen. Desforges[43] has demonstrated that oxidation of glutathione in normal red blood cells can be brought about by mere shaking in air. Glutathione may be a critical antioxidant compound in the red blood cell. It may protect the carbohydrate degrading enzyme chain or even hemoglobin itself. In the Helvey experiment, the bizarre cells seen in the 7.4 psi run and subsequent "demonstration of Heinz bodies upon incubation" are typical of the "Heinz body anemias" studied by Jandl et al.[96] These bodies are granules of precipitated oxidized hemoglobin and represent an apparent acceleration of red cell aging. The abnormal red cells in the other runs may, of course, possibly represent borderline oxidative damage. The finding of stippled cells makes one look again at the mercury toxicity problem, but let us not further confuse the issue at this point. The possibility of methemoglobin was mentioned in the oxygen saturation analysis. Methemoglobin is indeed an intermediate or a parallel reaction in the degradation of hemoglobin and the formation of Heinz bodies.[19, 21, 83, 96] The persistence of this anemia may indicate that not only the older cells, as in primaquine sensitivity, but also the younger cells are being damaged. An anemia lasting for about 100 to 200 days could be expected.

How can the blood picture in this experiment be reconstructed? The evidence is indeed strong for an "oxidative" hemolytic anemia. There is adequate animal and human experience outlined above to make an excellent case for it. Why has this not been reported in the past? The absence of nitrogen and possibly the addition of mercury toxicity or toxic oxidation products of the polyurethane-toluene-diisocyanate insulation of the liquid-oxygen pipes are the most obvious scapegoats. That sensitizing agents can do the trick is quite apparent. The matter is still open to question. The possibility that nitrogen acts as an intracellular antioxidative buffer is intriguing, but has less fact to back it. The buffering effect of nitrogen in the oxidative burning problem, discussed in Part II of this report, is an analogy that probably holds little water in this discussion. One could suggest that both animals and human red blood cells be exposed in bell jars with all combinations of the components of polyurethane insulation, mercury, and nitrogen-free 7.4 psi oxygen, and observed for the oxidative hemolytic interactions discussed above.

In last analysis of all the potential factors involved, it might be said that there was an oxidative hemolytic anemia with possibly a partial suppression of the reticulocyte response by high oxygen. In view of the well documented previous experimentation recorded here, it appears that the high p_{O_2} alone is probably not responsible for all of Helvey's findings.

In view of this potential oxidizing state, it would be well worthwhile to review the capacity of many drugs to cause methemoglobinemia. Acetanilid and acetophenetidin are the most obvious ones. Headache remedies containing them should be avoided. Bismuth subnitrate, commonly used in antidiarrhea preparations, should also be avoided, as should many aniline base compounds. Treatment of methemoglobinemia by ascorbic acid and methylene blue should also be reviewed. Hemoglobin electrophoresis should be used in routine screening of astronauts for "trait" conditions.

The Mercury Flights

In none of the recent Mercury flights have there been recorded any signs of general respiratory or hematological defects. Of course, the actual cabin exposures lasted for only a maximum of 9 hours. One would suspect that longer checkout periods on the ground have been attempted. There is no documentary evidence to substantiate this. There have been only rumors of X-ray signs of atelectasis in one of the Mercury astronauts. DuBois, in a memorandum to the members of Committee 16 of the National Academy of Sciences group on gaseous environment (March 8, 1962) mentions, "Physical examination of Shepard after recovery from his suborbital flight, revealed that he has moist rales at the bases of his lungs. These rales were interpreted as atelectasis. . . . Glenn has had atelectasis." A review of final NASA flight reports does not substantiate these findings. As will be discussed in Chapter 4, atelectasis could be expected under the conditions of these flights. Results of the recent ACEL-Johnsville study are also reported in Chapter 4.

SPECIFIC OXYGEN TOXICITY EFFECTS

Several recent human studies have pinpointed some of the side effects of high p_{O_2} environments. Daly and Bondurant [40] have studied the effects of oxygen on the cardioregulatory system. They find that breathing gradually increasing percentages of oxygen in air, from 20 to 100 percent, causes linear decreases in heart rate to about 90 percent of control rates. This is abolished by atropine and is accompanied by a rate-dependent decrease in cardiac output. The effect of these higher concentrations of oxygen on the myocardium itself was not ruled out.

Ernsting [53] has demonstrated recently that breathing 99 percent oxygen for 3 hours at sea level produces small but statistically significant decreases in apparent diffusion capacity and true diffusion capacity of the lung of humans but no change in the volume of blood in the capillaries and no change in total lung capacity. Concentrations of 50 percent oxygen (380 mm Hg) in nitrogen produced none of these changes. Whether the recorded changes were due to either increased resistance to diffusion by the alveolo-capillary membrane or a decrease in effective membrane area was not determined. More prolonged studies at concentrations from 50 to 100 percent would be helpful.

Sensitivity of the young human retina to oxygen toxicity is well known. The disease entity, retrolental fibroplasia, has been attributed to high oxygen tensions.[3, 67, 128] Current pediatric practice recommends that for premature infants with no signs of cyanosis, the oxygen in incubators be kept below 40 percent. Cyanotic children can, of course, tolerate higher tensions. This upper limit would have significance only when long-duration missions might involve this problem of the newborn.

There is some evidence that the adult human retina is adversely affected by oxygen, but only at high tensions. Behnke et al.[13] found progressive failure of peripheral vision and constriction of the visual field to 10° at 3 atmospheres, and this can occur as early as 4 hours.[46] Visual disturbances are most often the first central nervous system signs in OHP.

The effects of 100 percent oxygen on acute mental activity have recently been studied.[84] A complex audiovisual conflict test was administered to men exposed to 100 percent oxygen at 1 atmosphere for 3½ hours. There was no indication of impairment.

On a different tack, Dunn [50] recently checked the hypothesis that the gaseous nitrogen in air produces some degree of narcosis. He exposed subjects for 4 hours to atmospheres of constant

p_{O_2} (152 mm Hg) with decreasing p_{N_2} (down to 152 mm Hg), and also to constant p_{N_2} and increasing p_{O_2} (up to 608 mm Hg). He tested their complex coordination function on a multidimensional pursuit tester (CM 813E) at the U.S. Air Force School of Aerospace Medicine over a period of 24 hours in 4-hour periods, with apparently a return to room air between periods.

Dunn found that with constant p_{O_2} and changing levels of p_{N_2} from 152 to 608 mm Hg, there was no change in scores. As the p_{O_2} was raised, however, there was a significant decrease in fatigue rate of scores over a test period of 24 hours. Since Behnke et al.[15] showed that breathing 100 percent oxygen for 4 hours results in retention of 2 percent nitrogen in the body, the actual brain levels of nitrogen in the body at the end of each 4-hour period may have been higher than the threshold for denarcotization. At least a 9-hour denitrogenation should have been attempted to allow brain nitrogen to reach its minimum value.[57]

The decrease of fatigue with higher than normal oxygen at the same p_{N_2} had been reported in the past by Bills[20] and by Hauty et al.[86] Hauty suggested that concentration on the task "tends to cause hypoventilation" and the increased oxygen may compensate for it. The little excess oxygen in the plasma would hardly be expected to be a factor in this study, but is as good an explanation as any. The antifatigue factor may be worth remembering in the study of missions with long continuous work periods of this type.

Oxygen and Atelectasis

It is obvious from the discussion of animal and human experience that an environment with high p_{O_2} and low nitrogen has a tendency to produce atelectasis. The potential hazard of this condition in aerospace vehicles has recently been brought to notice by a series of reports.

ATELECTASIS IN FIGHTER PILOTS

In 1960 Ernsting[52] reported that RAF fighter pilots demonstrated coughing and breathlessness upon releasing their parachute harnesses and standing erect after flights on 100 percent oxygen. On occasion, deep, poorly localized chest pain was present. The bout lasted 10 or 15 minutes and, for any one individual, might vary in intensity from flight to flight. Moist sounds over the lung bases suggested fluid or alveolar collapse. X-ray signs of atelectatic lobules were in evidence; these cleared in 18 to 24 hours.

Ernsting postulated that the oxygen displaced nitrogen from the alveoli and was absorbed when spontaneous or acceleration-induced blockage of the bronchioles made the lobules a closed cavity. They also suggested that constriction of the lower chest by poorly fitting g-bladders tends to compress the lower lobes of the lung descending with the diaphragm under G_z loads and further aggravates this condition. Caro et al.[26] have studied the dangers in restriction of chest wall movement. Edema of the lower lungs from the engorged capillaries caused by G_z loads was probably also a factor. Ernsting pointed to the findings of McIllroy and Caro (personal communications with Ernsting) which indicated that release of tight strapping of the lower chest will result in spells of coughing.

In the report of Helvey[87] it was noted that one of the subjects experienced severe cough on deep inspiration required for determination of vital capacity. It appears that sudden release of collapsed alveoli or lobules can trigger a reflex cough and that Helvey's subjects had borderline atelectasis. Subsequent reports of similar aircraft experiences were noted by Langdon et al.[100] and Levy et al.[103] Evidence that acceleration forces play a prominent role in the mechanisms producing atelectasis was reported recently by Clark and Augerson.[32] They reported atelectasis in the posterior lung fields of subjects in "eyeballs-in" (G_x) acceleration while breathing 100 percent oxygen at a simulated 27,000-foot altitude.

There are rumors that the Russians have found atelectasis and cases of mediastinal emphysema in experimental animals exposed to high accelerations in low-pressure 100 percent oxygen. No published work has been found to this effect.

Doctors Wood and Helmholtz at the Mayo Clinic, in as yet unpublished experiments, have found cystic lung changes in dogs breathing air or 100 percent oxygen under 5g acceleration along the G_x vector. Posterior (dorsal) alveoli were filled with fluid; the more lateral alveoli were compressed but not filled with fluid, and the anterior (ventral) alveoli looked "dilated" more than "cystic." None appeared to be disrupted. No duration of acceleration was mentioned as being threshold for this pathology.

In human experiments, these investigators report one case of mediastinal emphysema in a subject who had previous experience on the centrifuge at accelerations higher than 5.5g (along the G_x vector), the acceleration which caused the present pathology. He had just started the 5.5g run, hyperventilating air, but not under the positive-pressure (40 cm H_2O) mask-breathing of air to which other humans had been exposed under similar conditions. He felt severe substernal pain which was diagnosed by X-ray as substernal emphysema and atelectasis. He has apparently suffered no further effects from this experience. None of the other human subjects experienced emphysema on air or oxygen, with or without positive-pressure breathing. Apparently, no studies were performed under reduced barometric environments.

ACEL-JOHNSVILLE EXPERIMENT

Dr. Crits of the U.S. Navy Aircrew Equipment Laboratory has presented the following information regarding recent unpublished experiments performed in conjunction with the Johnsville centrifuge group. Three men were denitrogenated and centrifuged with a g-profile similar to that of the Apollo mission. The subjects were then placed in a 5 psi 100 percent oxygen atmosphere and maintained in it for 14 days. They were then centrifuged along an Apollo reentry profile.

All subjects coughed during the first centrifuge run. In one subject atelectasis was seen by X-ray for several days after the first centrifugation. All subjects had a tendency to cough upon deep inspiration required for vital-capacity measurements. There were no changes in vital capacity in any of the subjects. There was a question of oxygen unsaturation of the blood in one or more of the subjects. There were no other respiratory or systemic signs or symptoms.

There was a slight drop in hemoglobins, but this also occurred in the controls. No abnormal red cells were seen, but Crits reports he was not especially looking for them. No signs of hemolysis were present. The drop in red cell count was attributed to "blood letting." Other laboratory studies were reportedly normal.

Dr. Crits feels that 5 psi at 100 percent oxygen is perfectly safe for the takeoff and landing and at least 2 weeks in the cabin. He does not feel that atelectasis is a problem which would interfere with a 14-day mission under this environment.

KAROLINSKA INSTITUTET EXPERIMENT

Barr[7] has recently reported results of centrifuge studies performed at the Laboratory of Aviation and Naval Medicine at the Karolinska Institutet, Stockholm. Subjects were exposed to $+G_z$ acceleration at 4.5g to 5.0g for several minutes, and arterial oxygen saturation was simultaneously recorded by continuous cuvette oximetry. With the subjects breathing air and wearing inflated anti-g suits, an immediate fall in arterial oxygen saturation was noted. After 1 minute of the first exposure, the oxygen saturation ranged between 95 and 81 percent; the arterial pH remaining unchanged. At the same time, respiratory minute volume increased. Repeated exposures caused the arterial saturation to fall at a faster rate and to a lower level with each run. The rate of resaturation was usually slow, and markedly so after several exposures. In several runs, subjects breathed 100 percent oxygen or did not wear g-suits. In most of these runs a limited, but nevertheless noticeable, fall in oxygen saturation occurred.

The arterial unsaturation is interpreted as a shunt effect with ventilation-perfusion defect caused by congestion and atelectatic collapse of alveoli in the dependent regions. The normal arterial pH levels were attributed to the compensation of hyperventilation hypocapnia by carbon dioxide retention arising from enlargement of the physiological alveolar dead space. Admixture of venous blood high in carbon dioxide content to the arterialized blood probably helped keep the pH normal. A rather complete discussion of the pathological physiology of acceleration, arterial hypoxemia, and atelectasis is presented.

BASIC PHYSIOLOGY OF ATELECTASIS

The actual physiological mechanisms involved in "oxygen" atelectasis have been known for many a year. The classic studies of Coryllos and Birnbaum[37,38] and of Henderson and Henderson[88] first suggested that the rate of development of atelectasis distal to an obstructed bronchus depended on the diffusibility of the contained gases, their chemical affinity for blood components, and their solubility coefficient. Oxygen and carbon dioxide are bound by hemoglobin and the blood buffer systems and so easily leave closed cavities. Air, containing nitrogen, is absorbed in about the same time as an equal volume of nitrogen, so nitrogen was pointed out as the "mechanical buffer" of the gases.

In a rather sophisticated study of the kinetics of absorption, Dale and Rahn[39] demonstrated that the gas with the smallest absorption coefficient controls the rate of lung collapse when the surface areas and the rate of blood flow through the pocket walls are constant. The formula for rate of absorption of gas is of interest in that it allows one to calculate the rate of collapse of any fixed body cavity filled with gases of known composition. Dale and Rahn's equation is given here with some modification to include other inert gases. Let

$\dot{Q}$ rate of blood flow through occluded lung
p_A partial pressure of alveolar gas in occluded lung
p_a partial pressure of gas in blood leaving occluded lung
P_v partial pressure of gas in mixed venous blood
$\dot{V}$ total volume absorbed per unit time
F fractional concentration of gas in occluded lung
α absorption coefficient of gas expressed as ml/liter of blood/mm Hg pressure difference
O_2, CO_2, N_2 the particular species
X any other molecular species present

If it is assumed that there is no diffusion barrier ($p_A=p_a$) and that Fick's law applies, then

$$\dot{Q}=\frac{\dot{V}F_{O_2}}{[(p_A)_{O_2}-(p_v)_{O_2}]\alpha_{O_2}}=\frac{\dot{V}F_{CO_2}}{[(p_A)_{CO_2}-(p_v)_{CO_2}]\alpha_{CO_2}}$$
$$=\frac{\dot{V}F_{N_2}}{[(p_A)_{N_2}-(p_v)_{N_2}]\alpha_{N_2}}=\frac{\dot{V}F_{X}}{[(p_A)_{X}-(p_v)_{X}]\alpha_{X}}$$

Then, since

$$\dot{V}=\dot{V}F_{O_2}+\dot{V}F_{CO_2}+\dot{V}F_{N_2}+\dot{V}F_X$$

the rate of lung collapse is

$$\dot{V}=\dot{Q}\{[(p_A)_{O_2}-(p_v)_{O_2}]\alpha_{O_2}+[(p_A)_{CO_2}-(p_v)_{CO_2}]\alpha_{CO_2}$$
$$+[(p_A)_{N_2}-(p_v)_{N_2}]\alpha_{N_2}+[(p_A)_X-(p_v)_X]\alpha_X\}$$

Thus, when the gases in the occluded lung have reached constant composition, the rate of collapse will be directly proportional to the blood flow of the occluded lung only if mixed venous blood conditions do not change. Typical figures for absorption coefficient (ml gas/liter blood/mm Hg pressure difference) determined by Dale and Rahn[39] were: $N_2=0.0185$, $O_2=3.5$, and $CO_2=4.0$. The affinity of red blood cells for oxygen and of the red cell and plasma buffers for carbon dioxide give these gases their high absorption coefficients.

TABLE 3.—*Solubility of Inert Gases in Water at 38° C* [AFTER ROTH [141]]

Gas	Solubility coefficient
Helium	0.0086
Neon	.0097
Argon	.026
Krypton	.045
Xenon	.085
Nitrogen	.013

The effectiveness of other inert gases as atelectasis "brakes" is of interest. The solubility coefficients of these gases in water are given in table 3.[141] The solubility coefficient for nitrogen in blood (0.0185) is greater than that in water (0.013) because of its greater solubility in red cell lipids. Helium and neon are much less soluble in lipids than are nitrogen and the other inert gases. It would thus appear that helium and neon would be better atelectatic brakes than nitrogen by a factor of about 2. Argon, krypton, and xenon would be less effective than nitrogen. It would be worthwhile to check these theoretical facts with actual experiments.

One-hundred percent oxygen would be expected to increase the rate of lung collapse. The time of collapse, according to the equation of Dale and Rahn discussed above, would be proportional to the number of molecules actually present in the alveoli; thus, a reduced pressure should hasten alveolar collapse. Actually, at 197 mm Hg, assuming $(p_a)_{H_2O}=47$ mm Hg and $p_{CO_2}=40$ mm Hg, the ratio of collapse time to that at sea level should be $\frac{197-(40+47)}{760-87}$ or 1/6. Rahn[136] calculated that the rate of collapse at 197 mm Hg should be 1/370th that at sea-level conditions. For the dog at least, Rahn calculated that it should take only 1 minute to completely collapse alveoli at 0.26 atmosphere (200 mm Hg). Table 4 demonstrates these experimental results and calculations. The relief of substernal distress in the subject of Welch et al.[166] by increase in p_{O_2} may have been initiated through this factor.

These factors would probably hold true for aural atelectasis in closed middle-ear cavities. As mentioned in Chapter 2, MacHattie and Rahn,[108] in their experiment with mice in environments of 100 percent oxygen at 197 mm Hg, observed that 20 of 115 mice died within the first 48 hours of exposure to this reduced pressure. The mice that died became typically inactive immediately after reduction in pressure and appeared to "sleep" most of the time until death. At the last stages they were humped up, fur erect, breathing in large gasps. On autopsy, their lungs showed complete atelectasis. In an interesting twist, four of the mice born under these conditions were removed for 2 hours to normal air and then returned to 100 percent oxygen at 190 mm Hg. They succumbed after 2 hours in this environment.

Progressive decrease in compliance in a series of rapid temporal tests in anesthetized dogs led Mead and Collier [111] and Finley et al.[58] to suggest that in the anesthetized experimental animal there is a distinct natural tendency for the lungs to collapse. A degree of atelectasis always exists at normal sea-level conditions. The results of Wu et al.[176] suggest that anesthetized man shows this same atelectatic tendency. After 2 hours of anesthesia, compliance of the human lung decreases to 65 percent of preanesthesia levels. Ferris and Pollard [56] have results which suggest that unanesthetized humans demonstrate the same tendency.

The "elasticity" of the lung and natural tendency of alveoli toward collapse has received much attention in recent years. Clements [33] in the Sixth Bowditch Lecture of the American Physiological Society reviewed the many recent studies on pulmonary surface active agents ("surfactants"). These proteolipid materials appear to be present in the liquid layer lining the alveolar walls. Theoretical considerations which treat alveoli as bubbles suggest that alveoli should normally collapse as a result of the surface tension of the liquid layer. Evidence is presented that this surface tension probably contributes up to two-thirds of the total "elasticity" of the lung which is measured in compliance studies. This normal collapse tendency is countered in healthy alveoli by a proteolipid material, rich in phospholipids, which reduces the surface tension of the liquid layer below that of plasma. There is probably a decrease in surface tension during expiration which keeps the alveoli from the complete collapse predicted if the alveoli were to act like ideal bubbles.

TABLE 4.—*Rates of Collapse of One Lung, With Airway Obstructed, When N_2 Is Present (Breathing Air) and When N_2 Is Absent (Breathing O_2)*[a] [AFTER RAHN[136]]

Gas	Rate of collapse		Time to collapse lung, min
	Observed, cc/min/kg	Relative	
Air at 1 atm	0.046	1	[b] 370
O_2 at 1 atm	2.87	62	6
O_2 at 0.26 atm	[b] 17.20	370	[b] 1

[a] After Dale and Rahn (1952, 1955).
[b] Calculated.

Recent work has been directed at an *in vitro* study of the surface active agent. The material is collected from dried pulmonary edema fluid of beef lungs. A surface-tension balance is used to study the relation of surface tension to surface area of water as modified by the lung extract. A hysteresis curve reminiscent of *in vivo* pulmonary compliance curves is obtained. Figure 7 represents such a curve with a control curve for water and a Tween detergent. Pulmonary extracts from infants with atelectatic tendencies appear to have defective curves with a tendency toward higher surface tension.

Pertinent to the oxygen toxicity problem is a recent study by Klaus, Kavel, and Clements on the stability of this surface active material. They demonstrated that only a phospholipid fraction of the material is capable of reducing tension in the surface balance apparatus. On standing, this activity disappeared. If nitrogen was substituted for air, the activity remained. Clements feels that pulmonary and other systemic effects of oxygen toxicity may be initiated by primary defect in phospholipid cellular membranes. The increase in atelectasis under conditions of high oxygen and low nitrogen may well be triggered by inactivation of the alveolar extracellular surfactant.

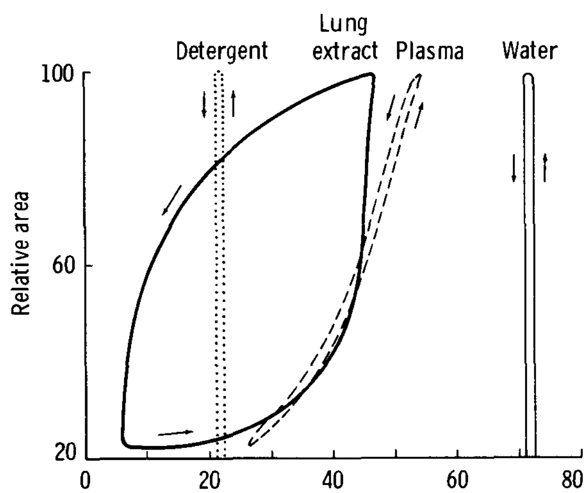

Figure 7.—*Surface tension-area diagram for water detergent and lung extract.* (After Clements.[33])

In a recent abstract, it has been noted that the lung fluids of rabbits exposed to high oxygen tensions do indeed have surface tensions higher than normal.[80] Preliminary results of work currently being carried out in the laboratories of Dr. Stuart Bondurant at the Indiana University Medical Center suggest that the sensitivity of surfactants in lung fluids of animals is quite species-specific. The surfactant of the rat, an animal generally resistant to oxygen toxicity, is resistant to changes by oxygen. Dogs, cats, and rabbits, which are sensitive to oxygen toxicity, appear to have surfactant which is sensitive to oxygen. This work is continuing and it should be of great pertinence to the atelectasis problem.

There appears to be an unsaturated lipid antioxidant in crude lung-extract powder which protects the powder from oxidation effects. There is also a trypsin-sensitive protein fraction which augments activity of the surfactant complex. All told, eight components have been isolated. They fall into three categories: an unsaturated phospholipid which reduces surface tension; a nonphosphated antioxidant lipid; and a protein skeleton holding the complex together.

This surfactant complex of the lung certainly deserves further study. Sensitivity of the material to oxygen *in vitro* and *in vivo* is documented. The possible use of synthetic aerosol surface agents during acceleration in high-oxygen environments at low pressure should be studied.

Thus, we have a picture of a lung that under ordinary air breathing is just about to collapse from "elastic tension" of the alveolar wall. The braking effect of nitrogen and surfactant ordinarily keeps the alveoli inflated. A shift to 100 percent oxygen, especially if at reduced pressure, increases the tendency to collapse by a factor of several orders of magnitude. When accelerations in the $+G_x$, $-G_x$, or G_z vectors are superimposed on this, the tendency to collapse is further increased by increasing local capillary pressure and alveolar fluid levels. If the lower chest is bound by g-bladders or a tight harness, the atelectatic tendency is still further increased.

What can be done to modify this potential hazard in space flight? The most obvious approaches are:

1. Encourage deep inspirations during periods of acceleration and at serial intervals throughout the stay in 100 percent oxygen environments.
2. During periods of acceleration, have subject on positive-pressure breathing to actually force the alveoli open.
3. Add inert gases to closed-circuit apparatus just before and during the acceleration maneuvers and revert to 100 percent oxygen for remainder of flight.
4. Maintain at all times in the cabin as high a percentage of nitrogen or other inert gases such as helium or neon as is compatible with proper oxygenation and cabin structural limitations.

The problems involved in each of these approaches will be discussed in Part III of this report.

CHAPTER 5

Combination of Oxygen Toxicity and Blast Effects

As DISCUSSED in Part II of this report, there is a danger in the blast effects which can be produced by penetration of meteorites. White and Richmond[167] have demonstrated that a major portion of the lethal effects of air blast on animals involves damage to the lung. The lung damage is seen especially after the "fast-rising" short-duration overpressure blast patterns to be expected from meteorite puncture.[109] The geometry of the cabin and distance of the subject from the wall are major factors determining pulse-wave geometry. What effect, then, do high oxygen tensions in the cabin atmosphere have on the original blast damage as well as on healing of lungs already damaged by blast?

WHITE-RICHMOND EXPERIMENTS

Fast-rising pressure pulses applied to the surface of the body can result in pulse waves traveling through the fluid phase at the velocity of sound in water (5,000 ft/sec). These pulses can reach the respiratory tree long before pressure pulses traveling at the speed of sound in air (1,000 ft/sec) can move down the airway to meet them. Recent work at the Lovelace Foundation suggests that the air pulse down the narrow respiratory passage is hardly a factor at all. Air, propelled by alveolar recoil, actually leaves the trachea. Hemorrhage from disrupted blood vessels, spallation, and implosion effects may damage the alveoli. If pulmonary blood vessels remain open to alveolar air, elastic recoil of the edematous, hemorrhagic, traumatized alveoli may "pump" emboli of gas into the arterial circulation.

It must be remembered that oxygen emboli following blast damage to the lung should be less dangerous than nitrogen emboli since absorption into the blood is more rapid. This would reduce the effective interarterial lifetime of any embolic bubble. After the lungs have ceased "pumping" emboli into the bloodstream, a reduction in p_{O_2} to almost hypoxic levels should accelerate absorption of the bubbles.

Low pressure of gas within the cabin should help reduce the original blast damage. The energy transfer from cabin wall to body wall should be attenuated by lower pressure. This would modify the fluid pressure wave impinging on the alveoli. Although the exact nature of this modification has not yet been adequately studied, it is expected that fatal overpressure hitting the body would be directly proportional to the total static pressure in the cabin. It is not expected that the small differences in molecular weight between nitrogen and oxygen would make a difference in the pressure wave. All that can be said at this point is that pure oxygen at reduced pressures within a cabin would probably be less harmful during the original blast episode than would mixed gases at higher pressures.

OHLSSON EXPERIMENT

The addition of oxygen at high tension to alveoli after blast was studied by Ohlsson.[122] He exposed rabbits to maximum pressure pulses of 10 kgf/cm² (about 10 atm pressure) with impulse values of 0.5 gmf/cm²/sec. Maximum pressure pulses of 14 kgf/cm² with impulse values of about 1.0 gmf/cm²/sec have 100 percent lethal effect on rabbits. Nine out of ten of the exposed rabbits survived the blast and transport to the chamber. All survivors were cyanotic; two showed dried blood around the nostrils. These animals were divided into two groups: five animals were placed in 80 to 90 percent oxygen at 1 atmosphere, and four were allowed to breathe air. The animals exposed to oxygen showed no improvement, all of them dying within 1 to 5 days with symptoms of suffocation. On the other hand, animals left in air showed, even after 24 hours, progressive improvement. Both the oxygen group and the air controls were sacrificed for histological study. The former group showed marked capillary exudation and fibrinous exudate even in nonhemorrhagic portions of the lungs. The latter had normal lungs in the nonhemorrhagic areas.

Ohlsson's explanation for poor response in oxygen in spite of cyanosis was that oxygen acts as a pulmonary vasodilator. This effect, first demonstrated by Euler and Liejestrand,[54] seems to have resulted in greater hemorrhage and exudation. In subsequent experiments in this same series, Ohlsson demonstrated that 3 percent carbon dioxide intensified the effect of oxygen toxicity on the blast damaged lungs of rabbits. Ohlsson concludes from these studies and others with diphosgene gas (no hemorrhage) that in cases of pulmonary damage with a hemorrhagic lesion there is a decreased oxygen tolerance and exacerbation of the lesion; in pulmonary damage with only hypoxemia, there is a greater tolerance for oxygen and actually demonstrable therapeutic value in high tensions of this gas.

OXYGEN THERAPY IN LUNG BLAST

From this one study it can be concluded that 80 percent oxygen at 1 atmosphere (p_{O_2}=610 mm Hg) will exaggerate the lung pathology in cases of blast injury. It would appear from the previous discussion that the atelectatic tendency would also be exaggerated in nitrogen-free atmospheres, with a resultant decrease in the effective alveolar area of the already damaged lung. Blast damage to the chest wall would also tend to predispose to atelectasis by restricting the depth of inspiration. It would appear that treatment of lung blast damage, even with the subject in a cyanotic condition, should not be attempted with p_{O_2} greater than 400 mm Hg. Preferably there should be nitrogen, neon, or helium present.

The entire problem of choosing a cabin atmosphere in light of the meteoritic blast potential is discussed in Part II of this report.

CHAPTER 6

Oxygen and the Space Radiation Problem

As DISCUSSED in Chapter 1, the effects of oxygen and ionizing radiation appear to be synergistic. The same free-radical mechanisms appear to be the damaging factors in both oxygen toxicity and some types of radiation damage; the same drugs tend to protect against both. What experimental work is available to give one a better quantitative feel for the synergism?

"OXYGEN EFFECT" IN RADIATION

The early studies of Thoday and Read[159] suggested that X-rays induced more chromosomal aberrations in the roots of the horsebean plant, *Vicia faba*, in the presence of oxygen than in its absence. It was subsequently found that this oxygen effect was dependent on the linear energy transfer of the radiation utilized. There was a larger oxygen effect with sparsely ionizing gamma rays and X-rays; a rather small one with more densely ionizing neutrons; and a negligible oxygen effect with the very densely ionizing particles. It appeared that there was a parallelism between the production of chromosome breaks and the formation of hyperoxal radicals and peroxides in water. Damage to molecules was, therefore, classified as (a) direct effects of ionization on bonds of the target molecule and (b) indirect effects via water free-radical systems.

A review of the direct effect on molecules has been presented by Wolff.[172] There is much evidence that the oxygen effect can work directly on the molecules as well as via the indirect water-hyperoxal mechanism, probably by an oxygen attack on the free radicals generated by the reaction

$$RH \xrightarrow{h\nu} R\cdot + H\cdot \qquad (1)$$

$$R\cdot + O_2 \longrightarrow RO_2\cdot \longrightarrow \text{Chain reaction} \qquad (2)$$

The functions of free-radical traps such as the thiols and AET have been discussed in Chapter 1. Several reviews of the early studies of the "oxygen effect" on the survival of animals exposed to X-rays and on protective compounds have been made by Gerschman et al.[63,64] These studies will not be described here in detail, for most involve the use of OHP rather than oxygen at less than 1 atmosphere.

Data from the study of Gerschman et al.[64] on the effect of the interval between radiation and OHP exposure are presented in table 5 and figure 8. The ordinates in figure 8 are differences in survival time in oxygen between mice previously exposed to X-irradiation and mice not exposed. The abscissae are the intervals between exposure to radiation and to oxygen. The shortest interval is 2 minutes. The animals remained in the high oxygen until death and the survival times were measured from the time 6 atmospheres were attained until the time of death. The maximal shortening caused by prior irradiation is 31.0 percent (series II in table 5). It may be seen in figure 8 that the oxygen must be administered within about 2 hours to be effective.

OXYGEN EFFECT AT <1 ATMOSPHERE

Little information is available on the effects of oxygen tensions at less than 1 atmosphere. In most of the recent therapeutic antitumor studies, OHP has been used to obtain the required augmentation of radiation effect.[29,64,143,146] This is because of the rather limited nature of the effect at lower oxygen tensions. Howard-Flanders and Wright[93] found that radiosensitivity of the growing bones of rat tails was

TABLE 5.—*Effect of Previous Radiation on Survival of Mice in High Oxygen Pressures With Varying Intervals Between Radiation and Oxygen Exposure* [AFTER GERSCHMAN ET AL.[64]]

Series	Interval	Number of experiments (a)	Atmospheres of oxygen	Sex	Mean survival time, min		Difference, min	Standard error of difference (b)	P, percent
					O_2	$r+O_2$			
I	Simultaneous	3	5	M	71.3	56.9	14.4	5.3	0.7
Ia	5 hours	2	5	M	65.0	59.0	6.0	6.4	34.8
II	2 minutes	2	6	F	49.1	33.9	15.2	2.8	0.0
III	30 minutes	3	6	F	44.5	39.1	5.4	2.3	1.9
IV	2 hours	4	6	F	35.9	31.4	4.5	1.9	1.8
V	5 hours	3	6	F	35.9	37.2	−1.3	2.3	56.9
VI	18 hours	3	6	F	40.9	42.8	−1.9	2.3	40.7

[a] In each experiment 20 mice were used, 10 irradiated and 10 controls. (Occasionally an observation on one mouse was missed.)

[b] Notes on the statistical analysis of the data: There was no evidence of heterogeneity of variance from one group of animals to another of the same sex. Males varied substantially more than females. Within any series of experiments there was no evidence of interaction between experiments and treatments. The standard errors for each sex are based on a pooled estimate of the within-group variance from all experiments with animals of that sex.

increased by a factor of only 1.3 when mice inspired oxygen at 1 atmosphere (no nitrogen) rather than room air. Gray et al.[78] reported a 50 percent increase in radiation sensitivity of Ehrlich mouse tumors under 1 atmosphere of oxygen. There have been several studies on the survival of mice under combined oxygen-irradiation exposures.[72] These papers were not available for review.

The Russians, as mentioned in Chapter 1, have been busy in this area. It would appear from table 6 that both 50 percent and 100 percent oxygen at 1 atmosphere reduced by 50 percent the percentage survival of animals exposed to 700 r; but these tensions have no effect on mean life span of the animals. One-hundred percent oxygen does counter the survival protection offered by AET (aminoethylisothiuronium), while 50 percent oxygen does not (series III). One can question the conclusion of these scientists that AET is not connected with the "oxygen effect." In these studies there would appear to be a threshold in this effect of oxygen in the range of 50 percent to 100 percent oxygen at 1 atmosphere. Studies of Gerschman [63] with OHP indicate a strong "oxygen effect" relationship with AET.

The "oxygen effect" as studied *in vitro* seems to be a confused issue. The diffusion problem encountered when chunks or slices of tissue are under study is apparently the key to varied results. Trowell [162] reports that *in vitro* explants of lymph nodes are 12 times as sensitive to X-radiation in 1 atmosphere of oxygen as in pure nitrogen. The sensitivity ratios for *in vitro* rabbit thymocytes and rat lymph nodes are in the 3 to 3.5 range for 100 percent oxygen vs nitrogen.[127] Attempts at quantitative study

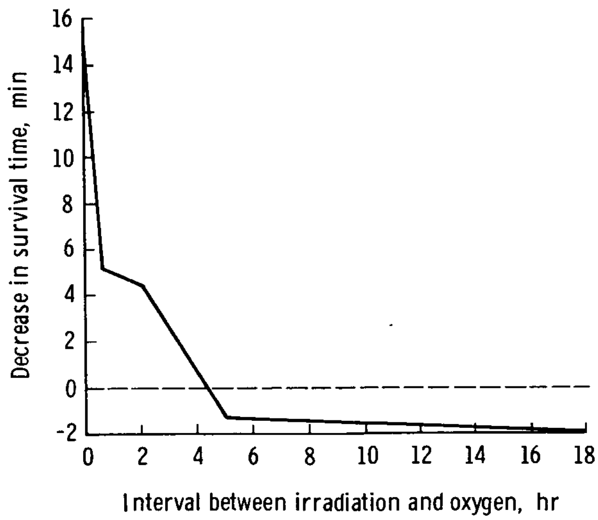

FIGURE 8.—*Effect of previous radiation on survival of mice in 6 atmospheres of oxygen with varying intervals between radiation and oxygen exposure.* (AFTER GERSCHMAN ET AL.[64])

TABLE 6.—*Effect of AET (10 mg/Mouse) on Survival of Mice Irradiated With Gamma Rays (Co^{60}), With Various Amounts of Oxygen in the Inhaled Gas Mixture* [AFTER GRAIEVSKII AND KONSTANTINOVA [76]]

Experimental conditions	Content of O_2, %	Survival		Mean life span, days
		Number ᵃ	Percent	
Series I, dose 900 r				
Control	21	0/30	0	5.1±0.4
AET	21	6/29	20.7±7.6	13.5±1.5
Hypoxia	8.2	14/29	48.4±9.4	10.2±1.3
Hypoxia+AET	8.2	25/32	78.1±7.3	15.1±2.9
Series II, dose 1,200 r				
Control	21	0/43	0	4.5±0.3
AET	21	1/41	2.4±2.4	5.3±0.4
Hypoxia	8.2	8/58	13.7±4.5	8.8±0.7
Hypoxia+AET	8.2	16/28	52.3±9.6	10.5±1.5
Hypoxia	6.5	15/47	31.9±6.8	14.1±1.4
Hypoxia+AET	6.5	20/35	57.1±8.4	23.3±1.3
Series III, dose 700 r				
Control	21	4/60	6.6±3.2	9.5±0.9
AET	21	10/55	18.1±4.7	13.9±1.0
Oxygen	50	2/60	3.3±2.3	9.2±0.7
Oxygen+AET	50	23/60	38.4±6.2	15.5±1.1
Oxygen	98.2	2/61	3.3±2.3	9.2±0.7
Oxygen+AET	98.2	13/58	22.4±5.5	11.2±1.0

ᵃ The numerator represents the number of mice that survived; the denominator, the number of mice in the group.

of the "oxygen effect" in specific organs *in vivo* are probably not clear-cut by virtue of poor monitoring of tissue p_{O_2}.

A Russian report reviewing thoroughly the "oxygen effect" has been published recently.[147] It substantiates the rather limited effect of oxygen on damage produced by radiation of high linear energy transfer, postulating that free radicals generated by an intense beam react with each other rather than with the peroxy free radicals of the water system. There were no data for high-energy proton radiation.

Gray et al.[78] have presented some interesting curves of the oxygen effect on sensitivity to X-rays and neutrons. Figure 9 shows how small an effect oxygen in the 20 to 100 percent range has on several different biological systems, even with X-rays of low linear energy transfer. Figures 10 and 11 show the oxygen effect with X-rays and neutrons on plant and animal systems. The data of Shchepot'yeva et al.[147] in table 7 show the effect of particles from radon on frog roe and tadpoles. "Oxygen water" contains about 30 mg of O_2 per liter of water; nitrogen water contains only 2 mg O_2 per liter of water. Lower survival rates in nitrogen water were reportedly not due to a "nitrogen effect" but due to the "less favorable dwelling conditions in nitrogen water." This was apparently evident in the controls without irradiation. Several other experiments with radon gas in water also demonstrated a lack of oxygen effect.

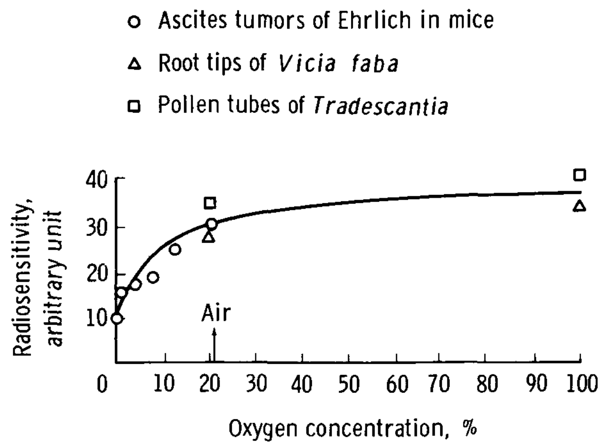

FIGURE 9.—*Dependence of radiosensitivity on oxygen concentration.* (AFTER GRAY ET AL.[78])

OXYGEN EFFECT AT 5 PSI 100 PERCENT OXYGEN

A most interesting, though only preliminary, study has been initiated by Dr. F. Benjamin[16] of Republic Aviation Corporation. He exposed 24 test and 24 control mice to 750 r X-irradiation in 100 percent oxygen at 5 psi and in air. The oxygen apparently increased the sensitivity of the mice. Figure 12 is a graphic representation of the results. Continuation of these studies appears vital to the solution of the present space-cabin problem.

It would thus seem that the oxygen conditions within space cabins should be a factor in consideration of the radiation hazard. The early

TABLE 7.—*Survival of Frog Roe and Tadpoles Subjected to Chronic Irradiation With Radon Solutions in "Oxygen" and "Nitrogen" Water* [AFTER SHCHEPOT'YEVA ET AL.[147]]

Concentration of radon, mache units	Water	Initial number of roe	Percent surviving after—				
			10 days	13 days	20 days	24 days	40 days
30 to 10^3	"Oxygen"	600	75	65	54	33	30
	"Nitrogen"	600	67	50	40	21	18
10^4	"Oxygen"	200	56	13	3	3	1
	"Nitrogen"	100	37	0	0	0	0
10^5	"Oxygen"	200	0	0	0	0	0
	"Nitrogen"	200	0	0	0	0	0

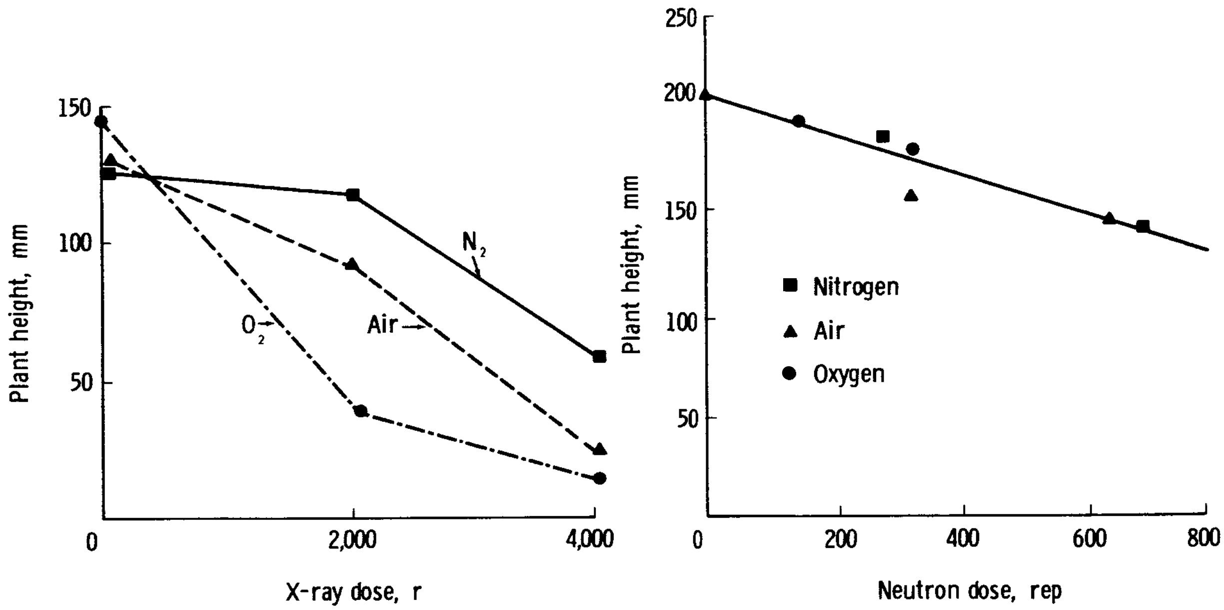

FIGURE 10.—*Development of barley sprouts irradiated with X-rays and neutrons under different atmospheric conditions.* (AFTER SHCHEPOT'YEVA ET AL.[147])

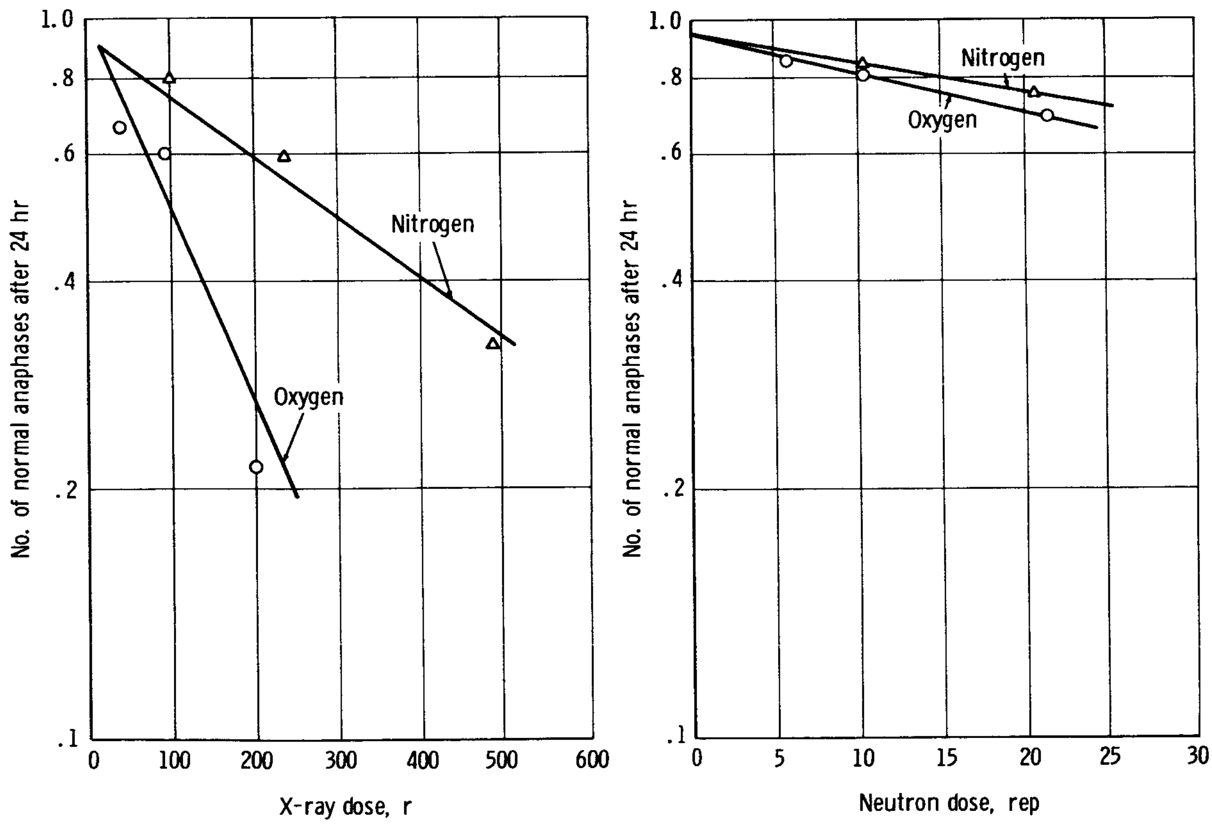

FIGURE 11.—*Radiation effects (cytological damage) in the cells of ascites tumors of mice exposed to X-rays and neutrons.* (AFTER GRAY ET AL.[78])

study of Thoday and Read [159] suggests, however, that for densely ionized proton or heavy primary radiation, the oxygen effect is less significant than for X-rays or gamma rays. The actual "oxygen effect" on these space radiations should be studied. There is no foolproof method of extrapolation from past work.

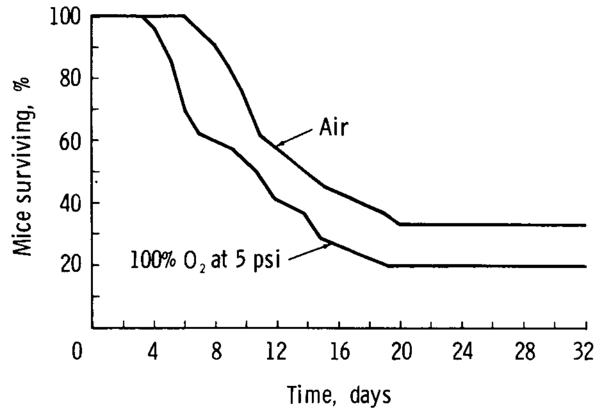

FIGURE 12.—*Effect of oxygen on sensitivity of mice to 750 r X-irradiation (preliminary results).* (AFTER BENJAMIN.[16])

CHAPTER 7

Drug Therapy and Protection Against Oxygen Toxicity

PHYSIOLOGICAL AND PHARMACOLOGICAL FACTORS

THERAPY against oxygen toxicity has for years been a confused issue. As was discussed in Chapter 1, most of the factors modifying oxygen toxicity operate at a general systemic level. All have control over energy metabolism. Mullinax and Beischer[117] have separated the factors into those that increase and those that decrease metabolic rate. Factors that decrease the metabolic rate and protect against oxygen toxicity are:

Starvation	Irradiation antagonists
Hypothermia	Hypophysectomy
Minimal activity	Adrenalectomy
Barbiturates	Thyroidectomy

Factors that enhance oxygen toxicity are:

Irradiation
Exogenous epinephrine
Exogenous thyroxin

It would appear that those conditions which can potentially decrease the rate of free-radical production in the metabolic process or trap free radicals intracellularly will decrease oxygen toxicity. The antiradiation thiol and thiuronium drugs as described by Gerschman[63] appear to offer the best hope for oxygen toxicity. A survey of these compounds has recently been made by Doull et al.[47] References to more esoteric drug effects may be found in the paper by Snapp and Adler.[149] Protective dosage effects of these drugs against oxygen toxicity in the <1 atmosphere range in humans have not yet been determined.

In view of the potential "oxidative" anemias suggested by Helvey's experiment, the use of methylene blue and ascorbic acid as agents against methemoglobinemia should be reviewed. These drugs are effective against the congenital methemoglobinemias caused by deficiency of diphosphopyridine nucleotide (DPN) diaphorase. As mentioned in the discussion of the Helvey experiment, those drugs predisposing toward methemoglobinemia should be avoided.

HYPOXIA, HYPOTHERMIA, AND RADIOSENSITIVITY

In the presence of potential radiation hazards such as solar flares, one might think of reducing cabin p_{O_2} to the 8,000-foot altitude level (p_{O_2}=120 mm Hg). It is well known that hypoxia will protect against irradiation. Dowdy et al.[48] showed that the dose of 250 Kev X-rays lethal for 50 percent of rats was increased from 600 r to 1,200 or 1,400 r by irradiating the animals in 5 percent oxygen at 1 atmosphere for 8 minutes. Histotoxic anoxia produced by NaCN gave no protection. This is as expected since tissue oxygen levels remain elevated in CN^- poisoning.

Wright and Bewley[175] demonstrated that the resistance of anesthetized mice to whole-body irradiation with 8 Mev electrons was increased by a factor of 2.3 to 2.5 when irradiation was initiated during the last 5 seconds of a 30-second period of breathing pure nitrogen. These investigators suggest that, in general, the protective effect of breathing nitrogen for short periods during irradiation gives animals a protection factor of 2.3 to 2.5, which is slightly higher than that produced by chemical agents but slightly lower than the factor of 2.8 achieved by hypothermia to 0° C.[92]

The Russian study by Graievskii and Konstantinova[76] indicates the protective effects of 8 percent oxygen and of carbon monoxide. The Russians have often spoken of hypothermia as a possible defense against space irradiation for long interplanetary missions and have initiated work along this line. A recent report by Milonov[115] covers this approach to the radiation problem.

It must be kept in mind that the densely ionizing "space" radiations which show a smaller oxygen effect may not be influenced by hypoxic states. Work should, therefore, be done to establish the actual degree of protection against this type of radiation which reduced p_{O_2} environments and low temperatures actually provide.

The problem of ozone generation in oxygen environments by space radiation will be covered in Part III.

INERT GASES AND OXYGEN TOXICITY

That the nature of the inert gas in a cabin may influence radiation sensitivity and oxygen toxicity has been demonstrated by Ebert and Howard.[51] These investigators have shown that very high pressures of hydrogen and nitrogen are protective of broad bean roots against high doses of X-ray in 1 atmosphere of air. Actually, it required 52 atmospheres of hydrogen to reduce the sensitivity to 70 percent of controls, and 107 atmospheres to reduce sensitivity to 55 percent of controls. Nitrogen at 20, 50, and 120 atmospheres reduces the radiosensitivity by only 40 percent. In the absence of oxygen, these gases had no effect on radiation sensitivity. Hydrogen was thought to tie up OH· free radicals. These start a chain reaction by attacking the R· radicals generated by the direct effect on the organic compounds. Nitrogen was postulated as displacing oxygen on the sensitive lipid-water or solid-water interfaces.

It does not appear that these experiments have any direct bearing on the requirement of inert gases in space cabins. The experiments of Helvey[87] which suggest such a requirement may, of course, be influenced by the "oxygen displacement" effects postulated by the Ebert and Howard experiment. It must be remembered that 1 percent nitrogen contaminating most experiments can "displace" 10 times as many oxygen molecules at critical sites as can the 0.1 percent nitrogen of the Helvey experiment. More work is obviously needed to support the idea that inert gases are not necessary over long periods of time for normal biological function.

At a recent American Rocket Society meeting, Schaefer,[144] in discussing oxygen toxicity, mentioned the requirement of nitrogen for embryological development. Reference was made to an abstract by Allen[1] of a study which discussed the toxic effect of 100 percent oxygen at 1 atmosphere on the development of chick embryos. Fertile hen eggs incubated at 1 atmosphere pressure in 20 percent oxygen and 80 percent helium showed the same retardation of development. "The addition of nitrogen to the extent of 10 percent of the partial pressure" was not sufficient to support adequate development. Fertile eggs incubated in 100 percent oxygen at a p_{O_2} of 150 mm Hg show the same lack of development of the vasculature as those incubated in 100 percent oxygen at 1 atmosphere. These results suggest that in the absence of gaseous nitrogen, or any other inert gas, the vascular system fails to develop even though the p_{O_2} is at normal levels. This work obviously needs followup, and Dr. Hiatt of Ohio State University has embarked on such studies.

The physiological aspects of inert gases will be more fully discussed in Part III of this report.

CHAPTER 8

Role of Oxygen Toxicity in Selection of Space-Cabin Atmosphere

THE TOTAL PROBLEM of selecting an optimum space-cabin atmosphere will be covered in Part III of this report. In this chapter, only the role of oxygen toxicity as a selection parameter will be discussed.

The review of human experiments has revealed no serious pathological states to be expected in 14-day missions using partial pressures of oxygen below 218 mm Hg (5 psi) with nitrogen concentration greater than 0.5 percent.

The studies of Helvey suggest that nothing is to be gained by going up to 7.4 psi. The "oxidative anemia" appeared much more severe at this pressure than at 5.0 or 3.8 psi. It is rather difficult to decide whether the anemia problem at 5 psi or below is severe enough to warrant exclusion of 100 percent oxygen atmospheres altogether. It is our feeling that a "sensitizing agent" was involved in these experiments. The lack of any sign of anemia in the experiments of Michel et al., Welch et al., and ACEL-Johnsville would suggest some other factor; however, it is possible that the low nitrogen content may play this role. If this were the case, "spiking" the oxygen with 1 to 2 percent nitrogen should solve the problem. The engineering details of this approach will be discussed in Part IV of this report. The renal problem is also probably related to a toxic factor other than high p_{O_2} or low nitrogen, though either of these two conditions may have potentiated this pathology.

More work is necessary to rule out the toxic or sensitizing agents postulated in the Helvey experiment. It would appear that 14-day studies are "cutting it too close." Thirty-day studies are more pertinent to the Apollo mission and will certainly be necessary for future missions.

On the low side of the pressure picture, it would appear from the review of the Dale-Rahn equations that 5 psi is a better choice than 3.8 psi. The lower the pressure of 100 percent oxygen, the sooner the onset of atelectasis. However, one factor not covered in past studies is the role of borderline bronchiolar pathology in rate of lung collapse. The less toxic bronchiolar reaction to high p_{O_2}, the lower potential hazard of bronchiolar obstruction initiating atelectasis. Experimentally, there was very little difference between the 3.8 psi and the 5 psi runs of Helvey in oxygen toxicity if anemia can be used as a criterion. It is also true that there was very little observed difference in the atelectasis problem between the static 3.8 psi and 5 psi groups. The 3.8 psi group demonstrated a greater drop in maximum breathing capacity during the last 4 days than did the 5 psi group, but diffusion capacity remained unchanged in both groups.

How serious is the atelectasis problem? At both 5 psi and 3.8 psi, subjects all seemed to be in a borderline atelectatic state. One may argue on theoretical grounds that their pulmonary reticuloendothelial mechanisms were also being taxed by oxygen toxicity, and their defense mechanisms against infectious disease were possibly also at a threshold. Clinically, however, no evidence of infectious disease was noted. Atelectasis does predispose humans to pulmonary infections. Infectious disease is always a problem in humans, but less so in "very healthy" individuals of the type that are being considered for space missions.

The transient "post-g" atelectasis of the

ACEL-Johnsville experiment and borderline atelectasis seen in many of the static runs were of such minor category as to eliminate them as a serious factor in predisposition to infectious diseases of the lungs. The nuisance factor of coughing falls into the same category as aural atelectasis, where the aerotitis and infectious otitis media factors loom much more important than the discomfort factor. Therefore, it does not appear that in the case of healthy astronauts, the infectious-disease potential or cough problem presented by the borderline atelectatic condition should rule out 100 percent oxygen environments. It also appears that on both theoretical and experimental grounds the 3.8 psi atmosphere is as safe as the 5.0 psi atmosphere as far as atelectasis is concerned.

If a decision had to be made only on the basis of hematological oxygen toxicity and atelectasis, it would seem that one pressure is as good as the other. The small increase in radiation sensitivity postulated for the "oxygen effect" in the case of densely ionizing proton and cosmic ray particles should not rule out 100 percent oxygen in the 5 psi range. The excessive toxicity of oxygen after lung blast should not influence the selection of an atmosphere in the 100 percent oxygen, 5 psi range. Both the radiation and lung-blast factors would favor the 3.8 psi atmosphere, but both would have less weight than the basic oxygen toxicity and atelectasis factors. If, indeed, a decision need be made today only on the basis of oxygen toxicity, atelectasis, space radiation, and physiological factors of lung-blast injury, a 4.5 psi cabin at 100 percent oxygen would probably be best choice. The many other factors in the final selection will be covered in Parts III and IV of this report.

It must be remembered that, at best, we are dealing with environments on the borderline of safety. If the evaluation of animal experiments is correct, there is a good chance that in studies of 30 days' duration or more, other signs of oxygen toxicity may crop up in man. In order to prepare for flights of long duration and to arm for the care of unusual symptoms in the 2-week flights, it seems that more animal and human studies are required. The following sections discuss areas which should be covered.

FREE-RADICAL CHAIN-REACTION MECHANISMS

It is obvious that much work needs to be done on the molecular basis of oxygen toxicity. Much of this area is being covered in the field of radiobiology. More coordination of effort and information exchange between respiratory physiologists and radiobiologists is needed. Rational approaches to drug therapy of oxygen toxicity requires more information on the free-radical problem. A thorough understanding of biological variability and combinations of toxic stresses in oxygen toxicity requires basic knowledge of free-radical oxidative mechanisms. The roles of lipid peroxides and thiols as reaction intermediates should be better defined.

The basic problem of the ultimate trigger for control of red blood cells appears to be related to the peroxide and free-radical problem. The role of intracellular peroxide levels in the kidney as the trigger for erythropoietic production should also be investigated.

OXYGEN TOXICITY IN ANIMALS

The unusual pathology which has presented itself in long-duration, borderline oxygen-toxicity studies in animals should be followed up. If other toxic, sensitizing, or even viral factors are key intermediates in the pathological process it is worth our efforts to find out exactly what they are. To say that they are "just experimental variables" is not enough. They are as important as "pure oxygen toxicity." Such findings in mice as hepatic degeneration, spastic paralysis, hemolytic (spleno-hemosiderosis) anemia, and acute fatal atelectasis should not be passed off as "experimental artifacts." Even the excess nitrogen loss in the urine of mice should be tracked down. Animals should be exposed to hot plastics and electronic equipment in environments of low nitrogen and high p_{O_2}.

OXYGEN TOXICITY IN HUMANS

The findings of Helvey, though they also smack of toxic or sensitizing factors, are significant. They suggest hitherto undefined combinations of toxic factors. The "bell jar" experiments with all combinations of low pressure, high p_{O_2}, low p_{N_2}, fresh polyurethane-toluene-diisocyanate plastic, and mercury vapor should be performed. The pertinent human experiments of the Helvey type should be repeated with no plastic or mercury factors for a period of 30 days at each of the three pressures. It is also apparent that the subjects in Helvey's last experiment should be closely followed until their blood and urine pathology disappears. They should then be followed intermittently for the rest of their lives. This should be done for the basic scientific information to be obtained and from a humane concern for their future health.

REQUIREMENT OF INERT GAS

The preliminary experiments of Allen which demonstrated failure of embryonic vascularization in 100 percent oxygen or low-nitrogen environments at normal p_{O_2} should be followed up. The use of inert gases other than nitrogen as atelectatic brakes should be studied. Other aspects of the inert-gas problem will be covered in Part III of this report.

OXYGEN AND ATELECTASIS

Experiments should be performed on the several proposed methods for avoiding atelectasis under high g loads. In these studies the $+G_x$, $-G_x$, and $+G_z$ vectors must be covered.

OXYGEN AND LUNG BLAST

The effects of low density, high p_{O_2}, and low nitrogen environments on the total body problem in air blast should be studied. Continuation of the Ling-Temco-Vought meteorite blast study with better control of physical parameters, physical measurements, body position, and atmospheric variables is urged. Also, the oxygen therapy of cyanotic lung-blast victims should be further studied. This is important for both the space and nuclear weapons problems.

OXYGEN AND RELIEF OF FATIGUE

There are now a few studies which suggest that elevation of the p_{O_2} above normal decreases fatigue factors in the psychological and complex coordination area. The mechanism is unclear, but empirical results appear well grounded. In view of the fatigue problems postulated for missions of long duration and complexity, it would be worthwhile to reevaluate these findings. Elevation of p_{O_2} in cabins during periods of fatigue may be of value.

OXYGEN AND SPACE RADIATION

It seems that current studies at the University of California and the Oak Ridge National Laboratory on effects of high-energy proton radiation should be extended to include the "oxygen effect." It appears that there will be little oxygen effect with this densely ionizing radiation. It would still be worthwhile to confirm the preliminary studies of Benjamin at Republic.

DRUGS AND OXYGEN TOXICITY

The emergency drugs used in the currently projected missions should be screened for their tendency to cause methemoglobinemia. The headache and diarrhea remedies containing acetanilid, acetophenetidin and bismuth subnitrate should be avoided. Animal studies should be initiated on the effects of oral methylene blue and ascorbic acid on oxygen toxicity of the borderline p_{O_2}, long-duration type. The effects of AET and the thiol drugs should also be studied in the low toxic p_{O_2} range.

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